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AGING IN APPARENTLY NORMAL MEN. II. ANDROGENIC ACTIVITY OF URINARY KETOSTEROIDS AND OF THEIR ALPHA AND BETA FRACTIONS; THE RELATIONSHIP OF ANDROGENIC TITERS TO VALUES OBTAINED BY COLORIMETRIC ASSAY*

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THIS series of reports pertains to the influence of aging upon certain aspects of the endocrine system and to the relation of the endocrine status, in turn, to various bodily structures and functions.

MATERIALS AND METHODS

Subjects consisted of 51 apparently normal men 21 to 75 years of age. An attempt was made to procure a group of comparable subjects with an extensive range of age, who were apparently normal and followed a similar routine of life. After preliminary surveys of several groups of persons, a series of prisoners was chosen for study. The means employed to avoid

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inclusion of subjects who were ill or physically unfit have been described elsewhere (1). Also listed in that publication are the extensive investigations which were carried out on these same subjects; the method of collection, extraction, separation, and measurement of urinary ketosteroids (KS);[1] and the values for colorimetric assays of total extract and for alpha[1] and beta fractions. Here it is sufficient to state that urine was collected for three to six days, the exact period of time being stated for each subject (1). Extraction and separation of KS followed the procedures employed by Dobriner and colleagues (3). Colorimetric measurements were made by the method of Zimmermann (4) as modified by Holtorff and Koch (5).

Bioassay of androgens was carried out on the combs of day-old chicks.[2] The precautions observed in caring for the chicks, and the procedures employed in inunction and in measurement of growth of the comb, have been described by Hollander and associates (6). Statistical validity was increased by following the principles of assay as outlined by Bliss (7). In brief, a total of 0.35 cc. of sesame oil, containing the extract to be assayed, was divided into seven equal doses which were applied daily for seven days to the comb of each chick. Animals were sacrificed on the eighth day, at which time the combs were excised and weighed. A total of 17 chicks was used for each of two dose levels of the extract to be assayed. Simultaneous tests were made at the time of each assay, with two dose levels (30 and 50 gamma per chick per week) of androsterone[3] as the standard androgenic preparation.

Dehydroisoandrosterone,[3] which is the source of most of the androgenic activity of the beta fraction in urine, was also used as a standard substance when beta fractions were assayed; therefore, in addition to the simultaneously derived standard curves for androsterone, dehydroisoandrosterone was used at two dose levels, 30 and 50 gamma per chick per week.

During the conduct of each assay 34 other chicks were kept as controls. The combs of 17 of these chicks were not inuncted. The combs of the other 17 animals were treated with 0.35 cc. of sesame oil without hormone. The oil was applied in seven equally-subdivided daily doses.

Androgenic activity is expressed in international units (I.U.). One I.U. is equivalent to the activity of 0.1 mg. of androsterone.

[1] The term "ketosteroids" is used in preference to the more commonly employed designation "17-ketosteroids." The first term is preferred because the methods employed recover compounds that include 3- and 20-, as well as 17- ketosteroids (2). Alpha-ketosteroids are the hydroxy compounds not precipitated upon the addition of digitonin to the neutral ketonic fraction. Beta-ketosteroids include hydroxy compounds that are precipitated upon treatment with digitonin.

[2] Chicks were obtained from Kerr Hatcheries in Frenchtown, N. J.

[3] Androsterone and dehydroisoandrosterone were obtained through the courtesy of Ciba Pharmaceutical Products, Inc., of Summit, N. J.

In both colorimetric and biologic assay the values for total extract refer to the sum of the values for alpha and beta fractions, which were measured separately.

Statistical analyses of all items were made in the form of 1) total correlations, which include men of all ages, and 2) partial correlations, in which the factor of age is minimized. The use of partial correlations reduces the possibility that two items, apparently related to one another, only seem to be so because both are related to the common factor of aging.

RESULTS

Comparison of androsterone and dehydroisoandrosterone as standard substances in androgenic assays

Average values for 5 simultaneously conducted androgenic assays of androsterone and dehydroisoandrosterone show lines with parallel slopes (Table 1). This indicates that the androgenic values for the beta fraction

TABLE 1. ANDROGENIC ASSAYS OF ANDROSTERONE AND DEHYDROISOANDROSTERONE, IN TOTAL DOSES OF 30 AND 50 GAMMA PER CHICK PER WEEK, GAVE AVERAGE DOSAGE-RESPONSE LINES WITH PARALLEL SLOPES. THESE AVERAGES ARE BASED ON 5 ASSAYS IN WHICH THE TWO STANDARD SUBSTANCES WERE TESTED SIMULTANEOUSLY. THE DATA REPRESENT COMB-WEIGHT ÷ BODY WEIGHT

Date of assay	Androsterone		Dehydroisoandrosterone	
	30 gamma	50 gamma	30 gamma	50 gamma
July 19–26, 1949	.00055	.00058	.00040	.00049
Nov. 8–15, 1949	.00052	.00061	.00040	.00052
Nov. 29–Dec. 6, 1949	.00058	.00087	.00035	.00055
Nov. 29–Dec. 6, 1949	.00063	.00080	.00042	.00063
Jan. 4–11, 1950	.00066	.00083	.00054	.00061
Average	*.00059*	*.00074*	*.00042*	*.00056*

may be calculated with either androsterone or dehydroisoandrosterone as the standard substance. The following calculations are based upon the use of androsterone.

Accuracy of methods

As reported previously (1), the possibility of error in the collection and preservation of urine, or in colorimetric assay of KS, was investigated by studies of duplicate collections of urine from 12 subjects. An interval of several weeks was allowed to elapse between the two collections. The agreement in values for the two samples was good; the average difference was ± 0.8 mg. of KS per twenty-four hours.

The errors in methods employed for bioassay of androgens are known to be large (8). This was found to be the case in the present study. Androgenic assays of the same alpha fractions from 4 subjects were repeated after an interval of fifteen months, with the results shown in Table 2. The generally lower values in the second test cannot be attributed entirely to loss of potency of the extracts, since greater activity was found in Case 42.

TABLE 2. A COMPARISON OF ORIGINAL VALUES AND THOSE OBTAINED UPON RE-ASSAY, AFTER ELAPSE OF AN INTERVAL OF FIFTEEN MONTHS, OF THE ANDROGENIC ACTIVITY OF THE SAME ALPHA FRACTIONS

Subject	I.U. of androgenic activity	
	Assay #1	Assay #2
#8	59.1	31.7
#21	16.0	11.0
#42	8.7	16.6
#44	18.4	10.1
Average	*25.6*	*19.9*

Thus duplicate bioassays of the same extract were in poor agreement, whereas colorimetric assays agreed well, even in different samples of urine separately extracted and assayed.

Because of the limited amounts of the extracts available for testing, the effect of cold weather upon two batches of chicks during shipment to the laboratory, and especially because of the rigid demands of the Bliss method of analysis, only an approximate range of androgenic activity could be determined in alpha fractions of 11 men and in beta fractions of 19 men. In these instances the androgenic values were estimated from the colorimetric assay of the KS in these fractions, but only values obtained by direct assay are used in the following analyses and graphs.

Influence of age on androgenic titers

Total extract (alpha plus beta). Figure 1 shows the values for individual subjects and a linear regression line for the decrease in androgenic titers with advancing age. The slope of the line is significantly different from zero ($P < .01$).

The average daily titer of urinary androgenic activity was 46 I.U. in 14 men 21 to 39 years of age, 24 I.U. in 13 men 40 to 59 years old, and 7 I.U. in 4 men 60 to 75 years of age. The decrease from the average titer of the young age group was significant at the 2 per cent level by middle age, and at or below 1 per cent by old age.

The progressive decrease in androgenic titers was noticeable even in terms of successive decades of life (Fig. 4). The average for the fourth decade of life is exceptionally large because of the unusually high value in one patient (Case 16), who excreted 117 I.U. per day, as compared with a mean of 32 I.U. by the other 5 men 30 to 39 years of age.

Alpha fraction. Figure 2 shows that daily titers of androgenic activity of the alpha fraction also decrease with advancing age. The slope of the linear regression line is significantly different from zero ($P < .01$) and is similar to that for the total fraction.

The average amount of androgenic activity per twenty-four hours was 43 I.U. in 16 men 21 to 39 years of age, 22 I.U. in 18 men 40 to 59 years old, and 7 I.U. in the 7 men 60 to 75 years of age. The decrease from the mean titer of the young men reached statistically significant levels by middle age ($P < .01$).

The progressive decrease in titers was noticeable in successive decades of life, as shown in Figure 5, except in the fourth decade. In this instance the exceptionally high titers in Case 16 again result in an average of 44 I.U. for the fourth decade as compared to a mean of 33 I.U. for the other 6 men 30 to 39 years old.

Beta fraction. Figure 3 shows that the mean androgenic activity of this fraction also decreases with age. There is a suggestion that the linear regression line may be significantly different from zero ($P = .04$), a suggestion supported by the fact that the difference between the average titer for the young age group and that for the combined middle and old age groups was statistically significant ($P = .01$).

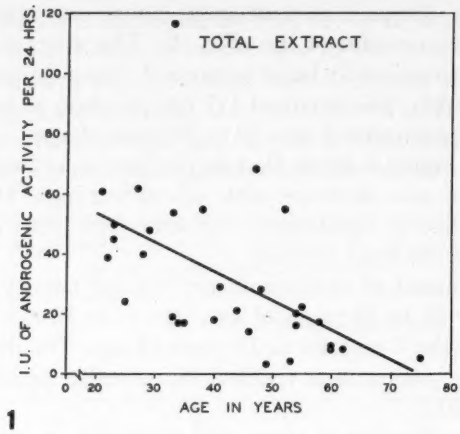
Average values for androgenic activity per twenty-four hours were 4 I.U. in 15 men 21 to 39 years of age, 2 I.U. in 13 men 40 to 59 years old and 1 I.U. in 4 men 60 to 75 years old. The average decline in successive decades of life is shown in Figure 6.

Proportion of beta to alpha fractions. There was no statistically significant change with age in the proportion of beta to alpha fractions, since the slope of the regression line was not significantly different from zero ($P = .30$).

The percentage of the total extract contributed by the beta fraction was 14 in androgenic assays, and 12 in colorimetric assays, both of which were carried out on the same 31 subjects.

Exceptionally high proportions of beta, as compared to alpha values were observed in the patients in Cases 26 and 30, in whom they were respectively, 11 and 24 times higher than the average of the values in the other 29 men studied in this regard.

Interestingly enough in Case 16, the man with the exceptionally high titers for total extract and alpha fraction, the proportion of beta to alpha fractions was within the normal range.



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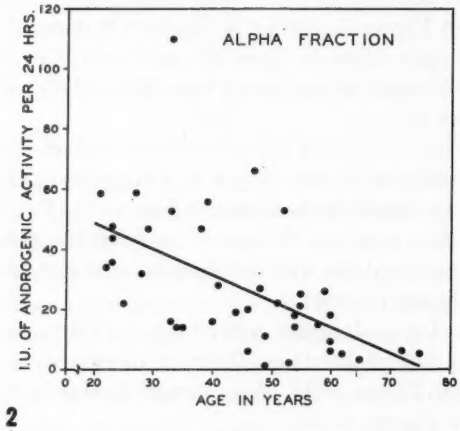

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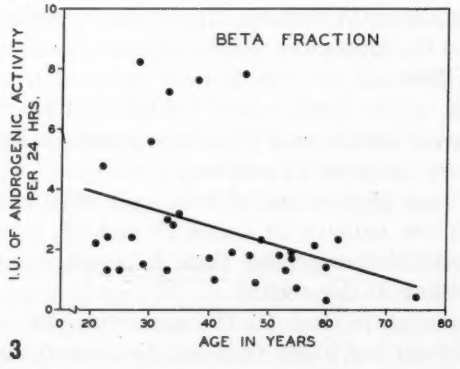

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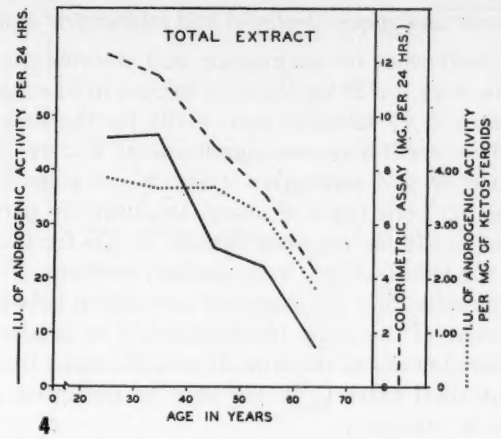
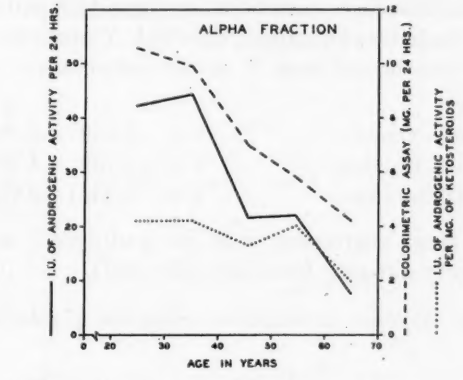
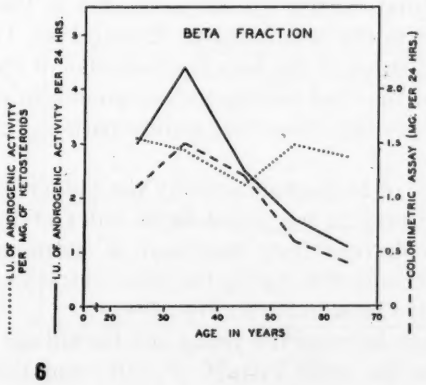
FIGS. 1 to 3. Daily titers of urinary androgenic activity of total extract and of alpha and beta fractions decrease with age, but the proportion of beta to alpha fractions does not change with age.


TOTAL EXTRACT
I.U. OF ANDROGENIC ACTIVITY PER 24 HRS.
COLORIMETRIC ASSAY (MG. PER 24 HRS.)
I.U. OF ANDROGENIC ACTIVITY PER MG. OF KETOSTEROIDS
AGE IN YEARS

4


ALPHA FRACTION
I.U. OF ANDROGENIC ACTIVITY PER 24 HRS.
COLORIMETRIC ASSAY (MG. PER 24 HRS.)
I.U. OF ANDROGENIC ACTIVITY PER MG. OF KETOSTEROIDS
AGE IN YEARS

5


BETA FRACTION
I.U. OF ANDROGENIC ACTIVITY PER MG. OF KETOSTEROIDS
I.U. OF ANDROGENIC ACTIVITY PER 24 HRS.
COLORIMETRIC ASSAY (MG. PER 24 HRS.)
AGE IN YEARS

6

FIGS. 4 to 6. Comparisons of the decrease with age in androgenic (biologic) and colorimetric titers, as shown in mean values per decade.

Correlation between androgenic (biologic) and colorimetric assays of KS

Correlation coefficients for androgenic and colorimetric assays in the same individuals were +.729 for the total extract in 31 subjects, +.708 for the alpha fraction in 41 subjects, and +.791 for the beta fraction in 32 subjects. All these coefficients are significant at $P < .01$. The correlation between colorimetric and androgenic values is not a spurious one due to the effect of age on both types of assay, as shown by partial coefficients of correlation of +.576 for the total extract, +.538 for the alpha fraction and +.757 for the beta fraction, with age held constant.

It is not remarkable that the degree of correlation between colorimetric and biologic assays of the alpha fraction should be similar to that of the total extract, since in tests of the same 31 men the alpha fraction comprised the bulk of the total extract, 90 per cent as measured colorimetrically and 92 per cent in bioassay.

Linear regression lines have been fitted to these data and are represented by the following predictive equations, in which Y gives the androgenicity (I.U. per twenty-four hours) and X is the colorimetric value (mg. per twenty-four hours):

For total extract:	$Y = -8.859 + 4.383\ X$
For alpha fraction:	$Y = -4.732 + 4.281\ X$
For beta fraction:	$Y = 0.3351 + 2.223\ X$

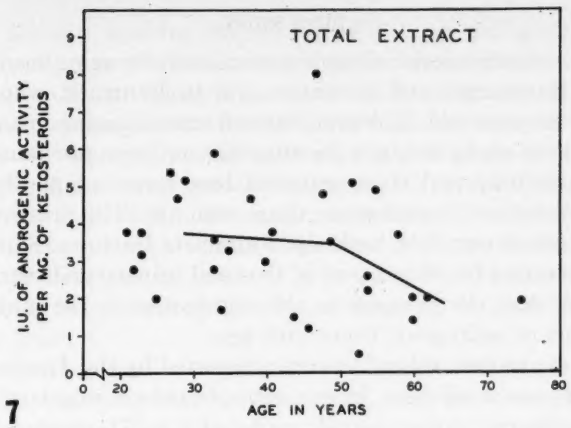
The slopes of these regression lines for androgenic and colorimetric data are significantly different from zero ($P < .001$).

I.U. of androgenic (biologic) activity per milligram of colorimetrically determined KS

The average number of I.U. per milligram of colorimetrically determined KS was 3.4 in the total extract (31 subjects), 3.6 in the alpha fraction (41 subjects), and 2.8 in the beta fraction (32 subjects). The lesser androgenic activity per milligram of the beta fraction than of the alpha fraction is due to the fact that the chief androgenic compounds in the beta fraction (9) tend to have less activity than their counterparts in the alpha fraction (9, 10, 11).

The number of I.U. of androgenic activity per milligram of colorimetrically-determined KS may be employed as an index of the proportion of urinary ketosteroids derived from testicular *vs.* extra-testicular sources (12). This index decreases with age in the total extract and in the alpha fraction, but not in the beta fraction (Figs. 7–9).

The decline in values between the young and the old age groups is statistically significant for the total extract ($P < .01$) and the alpha fraction ($P < .01$) but not for the beta fraction ($P > .50$).



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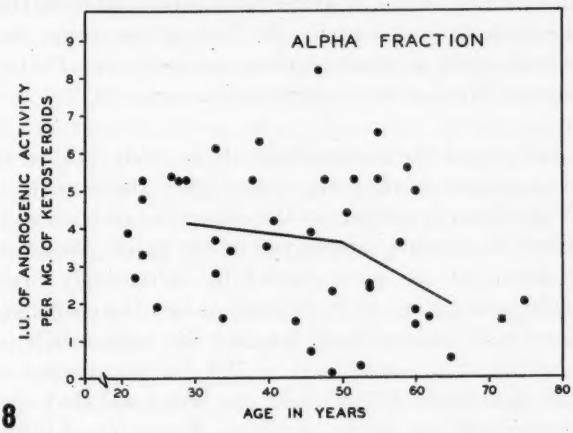

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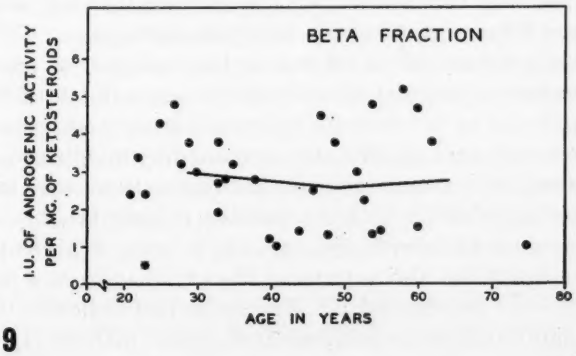

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FIGS. 7 to 9. An index, based on the I.U. of androgenic (biologic) activity per milligram of colorimetrically determined ketosteroids, decreases with age in the total extract and the alpha fraction, but not in the beta fraction.

DISCUSSION

The only extensive series of androgenic assays in aging men appears to be that of Hamburger and associates (13) in Denmark, who studied 62 males 20 to 80 years old. However, the influence of aging upon the androgenic activity of alpha and beta fractions has not been previously reported.

The Danish data and those reported here have comparable slopes in the curve of decline in androgenic titers with age. The present data show that this decrease occurs in both alpha and beta fractions. Since the alpha fraction accounted for 88 per cent of the total urinary androgenic activity, it is obvious that the decrease in this component is the major factor in the reduction of androgenic titers with age.

In terms of absolute values, the titers reported by the Danes are slightly higher then ours at all ages. In our subjects the colorimetric values were also slightly lower (1) than usually reported (14–17), presumably because "the food and routine of life in prison were unpalatable to the prisoners" who were the subjects in this study (1). This presumption was supported by the lean body-build of most of these subjects (18). Undernutrition is known to depress titers of ketosteroids in humans (19, 20) and in animals (21–23).

Detailed analysis of the composition of the body (18) in our series of subjects demonstrated beyond any doubt that the amount of body fat directly and significantly influences the quantities of both androgenic and nonandrogenic ketosteroids, as assayed in the urine. Progressively larger amounts of depot fat are accompanied by increasingly higher titers of ketosteroids. In young men, 21 to 39 years of age, the coefficient of correlation between the amount of body fat and the total urinary extract was +.844 for colorimetric assays and +.794 for androgenic assays. This relationship is significant ($P < .01$) and is a linear one that applies equally well to the very obese and to the very lean. For every additional kilogram of body fat the daily increase in titers amounted to 0.48 mg. in colorimetric assays of total KS, and to 3.2 I.U. in assays of androgen.

In middle-aged men (40 to 59 years) the coefficient of correlation between the amount of body fat and colorimetric assay of total KS was +.787 and was significant at or below the 1 per cent level. Androgenic titers and body fat were no longer significantly correlated by middle age. In old men (60 to 75 years) both colorimetric and androgenic titers were low and were not correlated significantly with the quantity of body fat.

There is a marked loss with age, not only in terms of absolute values for androgenic activity but also in terms of the I.U. of androgens per milligram of colorimetrically determined KS. The latter fact indicates that the proportion of highly androgenic compounds decreases with age. Low values for

androgenic activity, and for the number of I.U. per milligram of KS, are also found in other conditions in which testicular secretions are less than in young men (12), for example, in early childhood and in eunuchism.

The decrease with age in the proportion of highly androgenic compounds in the alpha fraction is of special interest, since this fraction contains the chief urinary metabolites of testicular secretions (11) and these metabolites account for the bulk of the androgenic activity of the alpha component (10, 11). Therefore, the pronounced decrease with age in the androgenic activity of this fraction, in terms of activity per milligram as well as in absolute values, is evidence of a decline with age in the secretion of androgens by the testes.

A less marked, but similar trend toward reduction with age in the urinary output of the highly androgenic compound, androsterone, has been observed in unpublished data which have been critically scrutinized by Doctor Thomas Gallagher of Sloan-Kettering Institute and made available to us through his courtesy. Some of these data have been published (11).[4] In 10 men, 21 to 35 years of age, an average of 3.3 mg. of androsterone (23 per cent of the alpha fraction) was isolated chemically. In 6 men, 61 to 76 years old, an average of 1.28 mg. of androsterone (18 per cent of the alpha fraction) was isolated. With regard to the two chief metabolites of testicular secretions, namely, the strongly androgenic androsterone and its weakly androgenic isomer, etiocholanolone, their proportions were practically unchanged by aging, being 42:58 in the young men, and 39:61 in the old men.

Thus there appears to be a decline with age in testicular secretions, analogous to that known for the ovarian secretions, but less abrupt and apparently less drastic.

Undiminished maintenance of adrenocortical secretions in aging men has been claimed (24) on the basis of assays of urinary neutral lipids. However, the data obtained in our study, analyzed elsewhere (12) with regard to this point, indicate that extragonadal contributions of KS precursors also decrease in aging men, but to a lesser extent than testicular secretions. The chief source of these extra-gonadal precursors is usually presumed to be the adrenal cortices (25, 26).

The testes continue their secretory function to some extent in old men, as shown by values for axillary hair, a secondary sex character that atrophies if testicular secretions are eliminated (27). The mean weight of this hair in men 60 to 75 years of age was still 37 per cent as much as that in

[4] After our manuscript had been submitted for publication, these data were published: Dobriner, K. The alpha-ketosteroid excretion pattern in normal males, *J. Clin. Invest.* **32**: 940–949, 1953.

men 21 to 39 years old (18). Further evidence of the continuation of some testicular function in old men is the fact that titers of urinary androgens are higher than in eunuchs (12). Since colorimetric values for total urinary KS are as low in old men as in young and middle-aged eunuchs (12), it can be deduced that in aging men the contributions to KS from extra-testicular sources must diminish. Otherwise, with the testes still functional, the values for urinary KS in old men would exceed those found in eunuchs. Still further support for this viewpoint is provided by isolation studies of 11-ketoetiocholanolone and 11-hydroxyandrosterone, compounds believed to be derived from adrenocortical secretions (11). This work, referred to previously and carried out at the Sloan-Kettering Institute, showed that 10 men 21 to 35 years of age and 6 men 61 to 76 years old had, respectively, average values of 0.92 and 0.68 mg. for 11-ketoetiocholanolone, and 1.21 and 0.92 mg. for 11-hydroxyandrosterone.

Reports in the literature state that androgenic activity can be reliably estimated from colorimetric values for total KS. Callow and associates (28) found a coefficient of correlation of +.745, and Zarrow and co-workers (29), +0.73. For the entire series of men in the present study the coefficient of correlation was +.729. This relationship is altered materially by aging, however, because of changes that occur in the alpha fraction and its androgenicity; apparently, then, aging men excrete a lesser proportion of the highly androgenic alpha-KS, androsterone.

Thus it is necessary to question the validity of the widely believed (5, 28–33) rule that androgenic activity of urinary KS can be reliably estimated from colorimetric assays. Further analysis (12) shows that this relationship is influenced not only by age but also by the sex of the individual and by the endocrine status. In fact the number of I.U. of androgenic activity per milligram of colorimetrically determined KS varies to such an extent with the degree of testicular function that it can serve as a reliable index of the relative contributions by testicular and extra-testicular sources to the urinary KS moiety (12).

Recent improvements of procedures employed in the extraction of dehydroisoandrosterone have led to the recognition that this substance is present in the urine of normal subjects in larger quantities (34–36) than revealed by the earlier techniques utilized when our work was carried out. Although our values for the beta fraction are lower than those obtained with later techniques, it is interesting that we found a decrease with aging, and this decrease was similar in proportion to that in the alpha fraction. It may be that whatever ketosteroids in the beta fraction were destroyed in our procedures (presumably some of the unstable compound, dehydroisoandrosterone), a similar effect was exerted in all urines in our study.

SUMMARY AND CONCLUSIONS

Aging in men is accompanied by a statistically significant decrease in titers of androgenic activity of urinary total extract and of the alpha and beta fractions. The mean number of I.U. per twenty-four hours in young (21 to 39 years), middle-aged (40 to 59 years) and old (60 to 75 years) men was 46, 24 and 7, respectively, for total extract; 43, 22 and 7 for the alpha fraction; and 4, 2 and 1 for the beta fraction.

Most of the decline with age in androgenic and colorimetric (KS) values was due to changes in the alpha fraction. This fraction accounted for 92 per cent of the total androgenic activity and 90 per cent of the total KS value. The proportion of beta to alpha fractions did not change with age.

Aging was accompanied by a significant decrease in the number of I.U. of androgenic activity per milligram of colorimetrically determined total ketosteroids (KS) and alpha-KS, but not beta-KS.

Diminished testicular secretions play an important role in the decline with age in both androgenic and colorimetric (KS) values, since most of this decrease is due to the alpha fraction which contains the chief metabolites of testicular secretions (11). One of these metabolites of testicular secretions, androsterone, accounts for most of the androgenic activity of the alpha fraction (10, 11). The marked decline with age in the alpha fraction, both in total androgenicity and in androgenic activity per milligram is, therefore, of significance. Other evidence supporting the conclusion that testicular secretions decrease with age is presented here and elsewhere (12).

The decrease with age appears to be considerably greater in contributions derived from precursors of testicular origin than in those derived from extra-testicular sources such as the adrenal cortices.

Estimations of androgenic activity from colorimetric assays had a correlation coefficient of +.723, but the reliability of this relationship shifts with aging and, as shown elsewhere (12), is influenced by the sex of the subject and by the endocrine status. The androgenic activity per milligram of colorimetrically determined KS also tends to increase with progressively higher daily titers of androgens.

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ADRENOCORTICOTROPIC HORMONE (ACTH) IN URINE*

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RELATIVELY adequate methods have been developed for assessing the changes in adrenocortical hormone production by urinary steroid assays. No comparable methods exist with respect to the detection of adrenocorticotropic hormone (ACTH) in urine. In view of the suspected importance of this hormone in states of stress such as surgical trauma, burns, excessive fatigue and irradiation damage, it becomes important for practical as well as theoretical reasons to have a ready means of assessing the varying concentrations of ACTH in body fluids. The availability of urine prompted our interest in a study of ACTH in this fluid, using the adrenal ascorbic-acid depletion technic in the hypophysectomized rat.

Since preliminary experiments involving the concentration of urinary ACTH by ethanol, benzoic acid, and by adsorption and elution from charcoal proved unsuccessful, and in view of the report by Williamson (1) that the injection of from 2 to 4 ml. of fresh urine from normal men and women into intact rats could be used in the detection of ACTH in human urine, we decided to undertake a study of urinary ACTH, using a technic similar to that of Williamson (1). Hypophysectomized rats were employed in preference to normal rats, since it is known that various noxious stimuli may produce a lowering of the adrenal ascorbic acid in intact animals by release of endogenous ACTH.

In the course of these investigations, it was found that spray-dried and lyophilized urines showed ACTH activity. Some assays on these materials are reported.

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ASSAY METHOD FOR ACTH

The assay method used in our laboratory was a modification of the Munson (2) technic, which is based on the adrenal ascorbic-acid depletion assay of Sayers, Sayers and Woodbury (3).

Hypophysectomized male rats weighing 90 to 110 grams were obtained from the Hormone Assay Laboratories in Chicago and used for the assay of ACTH on the third day after operation. ACTH preparations, usually dissolved in 1 ml. of 0.9 per cent sodium chloride, were injected intraperitoneally or intracardially. A control group of animals was injected with saline by the same route. One hour after administration of the hormone or saline, the animals were killed with Nembutal (12–15 mg.) and both adrenals excised. The excised adrenals were placed on moistened filter paper and fat and connective tissue carefully removed with a pair of fine eye scissors. A binocular loupe was used for magnification. The glands were weighed rapidly to the nearest 0.5 mg. on a Roller-Smith torsion balance, wrapped in cellulose acetate, sealed with Scotch tape, and stored at −15° C. until analyzed for ascorbic acid. Although analyses were not all carried out on the same day, glands from each experimental and the control group were analyzed simultaneously. At no time was there indication of loss of adrenal ascorbic acid during storage. Analyses of glands from different animals in a given group were sometimes separated by as many as ten days with no indication of a difference in ascorbic-acid content.

Ascorbic acid was determined by the method of Roe and Kuether (4), a standard curve being included with each day's determination. Ascorbic-acid concentrations are reported in micrograms of ascorbic acid per 10 mg. of fresh adrenal tissue (R_A). The mean values of R_A for each experimental group were compared with the mean values of the control animals in the same experiment, the difference between the means expressed as percentage of the control concentration, and the significance of the difference between the means of control and experimental animals determined on the basis of Fisher's (5) t value. With a total of 10 animals, t must be equal to 2.23 and for a total of 20 animals, t must equal 2.09, for the differences to be significant.

The quantities of ACTH in the various urines were calculated in those samples which produced a statistically significant decrease in ascorbic acid. The quantity of ACTH was determined from a log-dose response curve obtained with an Armour preparation (60–61). The values were converted to international units (I.U.) through the previously determined ratio of the potency of preparation 60–61 to that of the international standard, Armour LA-1-A. Those preparations which did not cause a significant depletion in adrenal ascorbic acid were considered to contain less than the minimum effective dose in the volume administered. The minimum quantities of ACTH that could be detected were 0.004 I.U. after intraperitoneal injection, and 0.00056 I.U. after intracardiac injection. The accuracy of the method as employed was better than ±40 per cent when 5 or more animals were used.

RESULTS AND DISCUSSION

Urines of normal individuals

Seven fresh urine specimens from 4 normal women and 8 from normal men, were tested for ACTH activity by intraperitoneal injection. The results of these assays are listed in Table 1. Significant decreases in adrenal ascorbic acid were caused by 4 of the 7 urine samples from women and 5

of the 8 urine specimens from men. The concentrations of ACTH in female urines ranged from less than 1.0 to 8.2 I.U. per liter. In male urines, the concentrations ranged from less than 1.0 to 3.1 I.U. per liter. When tests

TABLE 1. ACTH CONCENTRATIONS IN THE URINE OF NORMAL MEN AND WOMEN, AGED 20–40 YEARS

(4 ml. of fresh urine administered intraperitoneally)

Subject (Sex)	No. of hypophysect'd rats	Change in adrenal ascorbic acid (%)	Significance (t)	ACTH in urine (I.U./liter)
I (F)	5	−10	3.00	1.0
	9	−20	1.62	<1.0
II (F)	8	−47	4.12	8.2
	7	− 5	1.02	<1.0
	6	−30	4.28	3.1
III (F)	5	− 4	0.51	<1.0
IV (F)	7	−22	4.38	2.0
V (M)	8	− 3	0.54	<1.0
VI (M)	8	−12	2.38	1.1
VII (M)	8	−18	3.31	1.5
VIII (M)	7	−19	4.40	1.6
IX (M)	5	−13	2.05	1.2
X (M)	6	− 3	0.95	<1.0
XI (M)	6	−30	5.80	3.1

were made of several urines from 2 of the women (I and II), the concentrations were found to fluctuate. Subject I excreted amounts varying from less than 1.0, to 1.0 I.U. per liter. Subject II excreted amounts varying from less than 1.0, to 8.2 I.U. per liter.

Urines of patients subjected to stress

The ACTH contents of the urines of patients undergoing various types of surgery were studied. Six postoperative urines from women and 6 post-

operative urines from men, collected in the first twenty-four hours after operation, were assayed. The results of the assays are listed in Table 2.

TABLE 2. ACTH CONTENT OF URINE COLLECTED DURING FIRST TWENTY-FOUR HOURS AFTER OPERATION
(4 ml. administered intraperitoneally)

Subject (Sex)	No. of rats	Change in adrenal ascorbic acid (%)	Significance (t)	Urinary ACTH conc.	
				(I.U./liter)	(I.U./24 hrs.)
XII (F)	8	− 6	1.44	<1.0	—
XIII (F)	7	−16	4.50	1.4	—
XIV (F)	5	− 9	1.58	<1.0	<1.2
XV (F)	5	− 9	1.36	<1.0	<0.78
XVI (F)	4	− 6	0.91	<1.0	<1.5
XVII (F)	5	− 3	0.45	<1.0	<0.77
XVIII (M)	5	−15	3.22	1.3	—
XIX (M)	6	−15	2.18	1.3	—
XIX (M)	9	−57	6.06	15.0	—
XX (M)	9	−53	5.52	12.0	—
XXI (M)	8	−19	4.54	1.6	—
XXII (M)	8	−23	5.50	2.0	—

Four quantitative collections of urine were made from females. The ACTH concentrations varied from less than 1.0, up to 1.4 I.U. per liter. The activity in the 4 urines collected quantitatively was below the sensitivity of the method, but none of the women excreted more than 1.5 I.U. per twenty-four hours.

No quantitative collections of urine were made from males. All 6 of the urines showed activity at 4 ml., the quantitative evaluations ranging from 1.3 to 15.0 I.U. per liter. In these postoperative urines, the high values may have been the result of oliguria rather than of an increased rate of ACTH excretion.

Lyophilized urines from ACTH-treated children with rheumatic fever

In Table 3 are listed the results of quantitative determinations of the ACTH excretion of 3 children receiving varying amounts of ACTH for rheumatic fever. Urine volumes of 4, 8 or 16 ml. were injected for assay. The first patient, XXIII, excreted 0.53 I.U. of ACTH per twenty-four hours in a control urine assayed by intracardiac injection, and less than 0.33 mg. in another control urine assayed by intraperitoneal injections. No significant increases in urinary ACTH could be demonstrated after 20,

TABLE 3. URINARY EXCRETION OF ACTH BY CHILDREN WITH RHEUMATIC FEVER, BEFORE AND DURING ACTH TREATMENT

(lyophilized urine concentrates)

Patient (Age-Sex)	ACTH admin. per day, LA-1-A equiv. (mg.)	Vol. urine admin. (ml.)	Route of admin.*	No. of hypoph-ysect'd rats	Change in adrenal ascorbic acid conc. (%)	Signifi-cance (t)	Urinary ACTH conc. (I.U./24 hrs.)
XXIII (10-F)	0	16	IP	5	− 2.7	0.43	<0.33
	0	4	IC	7	−15.1	4.65	0.53
	20	16	IP	3	−18.0	7.70	0.18
	20	16	IP	8	− 7.7	2.70	0.26
	30	8	IP	2	−22.0	—	0.48
	80	16	IP	6	+ 1.0		<0.48
XXIV (11-F)	0	16	IP	6	+ 3.4		<0.13
	10	16	IP	7	− 4.3		<0.35
	40	16	IP	5	+ 2.0		<0.39
XXV (13-F)	0	16	IP	6	−13.3	1.61	<0.26
	20.8	8	IP	5	− 1.4	0.18	<0.27
	20.8	8	IP	4	− 8.8	0.97	<0.30
	24	8	IP	6	−22.4	2.71	0.70

* IP = intraperitoneal.
IC = intracardial.

30, or 80 I.U. of daily intramuscular doses of ACTH. The second patient, XXIV, excreted less than 0.13 I.U. per twenty-four hours in a control urine. Since the urine volume increased greatly after administration of 40 and 10 mg. of ACTH, a 16-cc. equivalent of urine was administered intraperitoneally; however, no significant decrease in ascorbic acid could be demonstrated. Therefore, in spite of treatment, daily levels equal to 0.39 I.U. were not reached. The last patient, XXV, excreted less than 0.26 I.U. in a control urine. After treatment, the excretion rose on one occasion to 0.70 I.U. per twenty-four hours.

Urines of patients with Cushing's syndrome

Urines from 2 women with Cushing's syndrome were assayed quantitatively (Table 4). One patient, XXVI, excreted ACTH at the rate of 5.7 and 10.4 I.U. per twenty-four hours. This is a much higher level than was seen in normal subjects or in the postoperative patients. This patient was found at autopsy to have a benign adrenal tumor. The other patient, XXVII,

TABLE 4. ACTH IN URINE OF PATIENTS WITH CUSHING'S SYNDROME

Patients	Amount of urine (ml.)	Route of admin.	No. of hypophysect'd rats	Change in adrenal ascorbic acid (%)	Significance (*t*)	Urinary ACTH conc. (I.U./24 hrs.)
XXVI	2	IP	6	−28	3.75	10.4
	4	IP	6	−28	2.37	5.7
XXVII	1	IC	7	−14	2.33	0.91
	2	IP	7	0	—	<2.4
	4	IP	7	− 2.3	0.74	<1.2

had been excreting cortin at the high rate of 2.9 I.U. of 11-dehydrocorticosterone equivalent (biologic assay) or 2.8 I.U. of desoxycorticosterone equivalent (chemical assay) per twenty-four hours when examined three months earlier. No ACTH assay was carried out at that time. Urinary ACTH was later determined at a time when the cortin excretion (chemical) was normal and clinical improvement was noted due to a spontaneous regression. Urinary ACTH excretion was 0.91 I.U. per twenty-four hours, a rate similar to that seen in the normal subjects.

Spray-dried pregnant mare's urine

A preparation of spray-dried pregnant mare's urine (kindly supplied by Dr. S. Gordon, Endo Products, Inc.) was assayed for ACTH activity by intraperitoneal and subcutaneous injection. Statistically significant decreases in adrenal ascorbic acid were found when doses of 80 to 100 mg.

TABLE 5. ACTH IN VARIOUS URINES

Source of urine	No. of assays	Range of ACTH concentration	
		I.U./liter	I.U./24 hrs.
Pregnant mare (spray-dried)	7	2.6– 3.0	
Normal women (aged 20–40 yrs.)	7	<1.0– 8.2	
Normal men (aged 20–40 yrs.)	7	<1.0– 3.1	
Women—after surgery	6	<1.0– 1.4	<0.77-<1.50
Men—after surgery	8	<1.0–15.0	
Cushing's disease			
active	1		5.7; 10.4
inactive	1		0.91
ACTH-treated children with rheumatic fever (lyophilized urine)	14		<0.13–0.70

were administered. In 3 separate experiments, adrenal ascorbic-acid decreases ranging from 13.9 to 18 per cent were found, with t values ranging from 3.50 to 4.66. On the basis of these results, the titer of urinary ACTH was found to be between 2.6 and 3.0 I.U. per liter.

Table 5 summarizes the urinary ACTH content of human and of pregnant mare's urine.

SUMMARY

Adrenocorticotropic hormone (ACTH) activity has been detected in the urine of normal men and women by means of the intraperitoneal and intracardial administration of fresh urine to hypophysectomized rats. Normal men (20 to 40 years of age) excreted between <1.0 and 3.1 I.U. of ACTH per liter, and normal women (20 to 40 years of age) excreted between <1.0 and 8.2 I.U. per liter. There was a tendency to increased ACTH excretion during the first postoperative day in surgical patients. No increased excretion of ACTH could be demonstrated in patients with rheumatic fever, following the administration of 20 to 80 I.U. of ACTH per day.

One patient with active Cushing's disease showed a five- to ten-fold increase in ACTH excretion above the normal level, whereas a second patient whose disease was inactive excreted an amount of ACTH similar to that of normal subjects.

A spray-dried preparation of pregnant mare's urine contained a significant amount of ACTH.

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THE CASE FOR CORTICAL STEROID HORMONE ACCELERATION OF NEOGLUCOGENESIS FROM FAT IN DIABETIC SUBJECTS. A SUMMARY OF FIVE YEARS' INVESTIGATIVE WORK*

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PRODUCTION or accentuation of the diabetic state by adrenocorticotropin and by cortical steroids is well documented (1). In association with the diabetogenic effect of these hormones, one also observes a progressive degree of insulin resistance. The mechanism of these metabolic changes has never been satisfactorily explained.

The possible explanations for such effects include the following:

1. *Inhibition of insulin production.* Since corticotropin and cortical steroids accentuate the diabetes associated with total pancreatectomy, this is obviously an inadequate explanation.

2. *Insulin destruction.* Unsatisfactory, as in "1."

3. *Blockade of insulin effect,* perhaps at the enzymatic level. On the basis of recent findings in our laboratory, this appears probable (2). As in "1" and "2," however, such an hypothesis fails to explain the *total* diabetogenic effect of adrenal steroids.

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4. *Acceleration of neoglucogenesis from protein.* This effect is well documented, as described subsequently.

5. *Acceleration of neoglucogenesis from fat.* Such an effect need be postulated only if neoglucogenesis from protein is inadequate to explain the total neoglucogenesis resulting from steroid hormone administration. We believe that this is so (see Experimental Section).

The studies which follow, some of which have been reported in abstract form elsewhere (3), were designed to prove or disprove the concept of acceleration of neoglucogenesis from exogenous and/or endogenous fat by adrenal steroids in human diabetic patients.

EXPERIMENTAL PROGRAM

It has been demonstrated that corticotropin and adrenal steroids diminish or prevent fasting-induced hyperketonemia in nondiabetic subjects (4). Initially, therefore, fasting per se was used as the basic experimental technique in diabetics. Under fasting conditions, the levels of blood and urinary sugar, ketones, and urinary nitrogen were determined in diabetic subjects during control periods and during the administration of ACTH and/or cortisone.

Subsequently patients were studied for more prolonged periods of time, during which they received quantitatively constant formula diets containing only protein and fat. In the course of these studies, the investigative program was expanded to include determination of respiratory quotients, plasma and urinary sodium and potassium, chlorides and CO_2, and at times urinary and plasma phosphorus, magnesium, sulfur, and other constituents of protein tissue.

Methods

Except for calcium and magnesium determinations, the methods used have been described in previous papers (4, 5). Urinary and fecal calcium and magnesium were determined by the method of Michaels *et al.* (6).

EXPERIMENTAL FINDINGS

Study 1. The first patient studied, HAR, was a young female with diabetes of moderately severe degree. Her insulin requirement on an average diet was 60 units per day. In the course of her "control fast" the blood sugar, after an initial fall, rose from levels below 100 mg./100 cc. to values in excess of 350 mg./100 cc. at the end of three days (Fig. 1). The ketones rose from values of approximately 2 mg./100 cc. to levels of 85 mg./100 cc. within a 72-hour period. Sugar excretion during the three days of the control fast totaled 40.58 Gm. and urinary ketones totaled 18.26 Gm. The urinary nitrogen averaged approximately 8 Gm. per twenty-four hours.

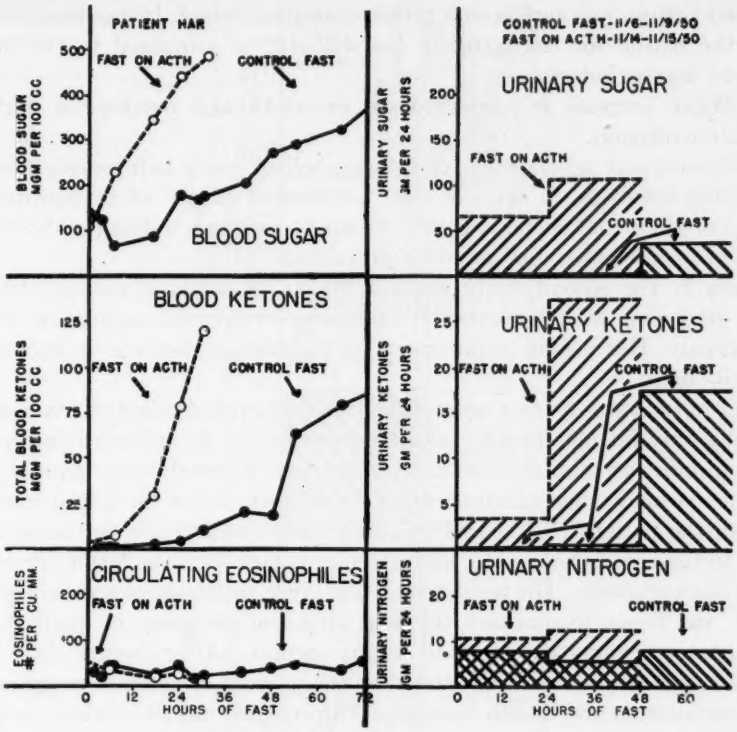

FIG. 1. Accentuation of fasting-induced hyperglycemia, hyperketonemia, glycosuria and ketonuria by ACTH in diabetic Patient HAR.

During the second fast, ACTH was administered intramuscularly in a dosage of 25 mg. every six hours. Thirty hours after ACTH administration was begun, the blood sugar level was 489 mg./100 cc. and the urinary ketones 120 mg./100 cc. The patient was clinically severely acidotic, and required several hundred units of insulin during the next few hours to control the hyperglycemia and ketoacidosis.

During the first twenty-four hours of the fast, she excreted large amounts of sugar and ketones, and there was a tremendous increase in the latter during the second twenty-four hours. (The second 24-hour period cannot be interpreted quantitatively, because of the administration of some glucose.) Nitrogen excretion was significantly increased as compared to the corresponding period of the control fast.

From the foregoing, it was apparent that at least in this diabetic, ACTH caused rapidly progressing and extreme hyperglycemia in association with insulin resistance and dehydration; and further, that the fasting-

induced ketosis was accelerated rather than diminished. It was postulated that the reason for the latter in the diabetic as compared to the nondiabetic was as follows:

1. Major increase in carbohydrate excretion and decrease in carbohydrate oxidation.

2. Consequent acceleration of fat catabolism, with ketogenesis vastly exceeding ketolysis, in spite of the "antiketotic effect" of corticotropin. It was obvious that such studies could not be pursued, unless the development of insulin resistance could be prevented.

Study 2. The second study was carried out in a female patient, BRO, aged 40 years, with diabetes of moderate severity in association with acromegaly. Her insulin requirement on an average diet was in excess of 40 units daily.

This patient was given a quantitatively constant formula diet containing initially only fat plus protein, and finally only fat. As indicated in Figure 2, no insulin was administered until progressive hyperglycemia and hyperketonemia made such administration mandatory. After the initial control period, the patient received successively and cumulatively cortisone acetate, testosterone propionate, and adrenocorticotropin, the latter administered intravenously. The testosterone was given to minimize protein catabolism, and hence to diminish this as a source of confusion in qualitative-quantitative evaluation of total neoglucogenesis. After twenty days the quantitative study was discontinued on this patient, because of progressive hyperketonemia and insulin resistance. During this time the patient showed negative nitrogen and phosphorus balances, and for part of the time, despite an increase in potassium intake, she had a negative potassium balance.

It will be noted that in this patient, as in the fasting diabetic, neither ACTH nor cortisone adequately suppressed hyperketonemia. It was again postulated that the development of insulin resistance (with consequent decrease in carbohydrate oxidation and increase in fat catabolism) was the largest single factor responsible for the different responses to ACTH and cortisone between the diabetic and the nondiabetic subject, in regard to ketone metabolism.

Study 3. The next study was performed in BUN, a female patient aged 42 years, with relatively mild diabetes. Throughout the study, during which time she was maintained on a chemically constant formula intake supplemented with vitamins and essential minerals, she received no insulin. Throughout the portion of the study shown, she received testosterone propionate, 100 mg. intramuscularly daily, to minimize net protein catabolism and to conserve potassium. Additional amounts of potassium and/or

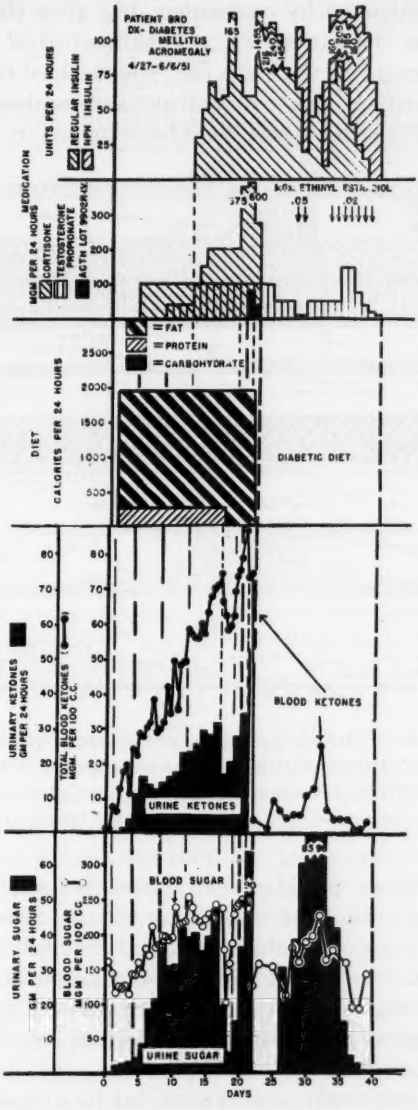

FIG. 2. Accentuation of diabetic state and of insulin resistance by cortisone and ACTH in diabetic-acromegalic Patient BRO. A total of nearly 1,500 units of insulin was required to control the effects of intravenous ACTH.

sodium were administered by continuous drip after the first twenty-four hours; nevertheless, the patient was not maintained in potassium equilibrium. From the second to the sixth day, she received 600 mg. of cortisone daily and intravenous ACTH in an amount varying from 40 to 60 mg. per day, as shown in Figures 3a and 3b. The response to the administration

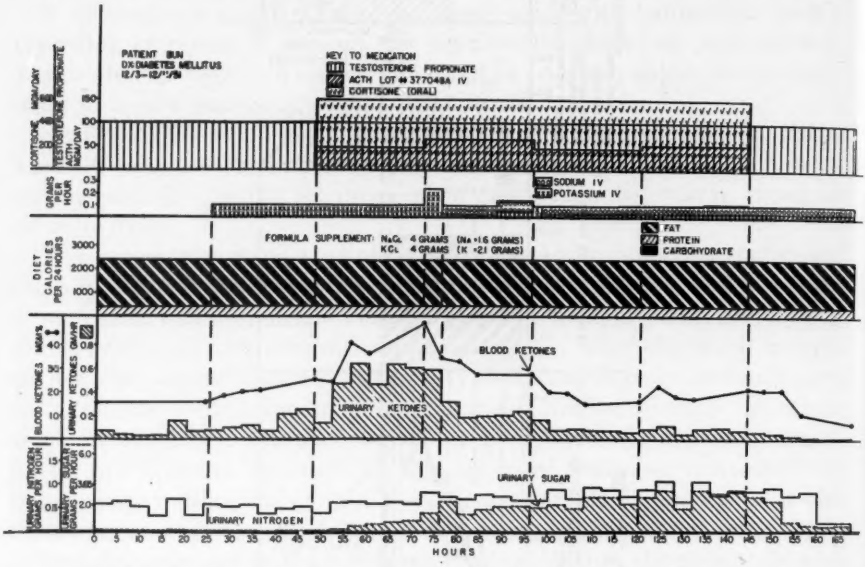

FIG. 3a. Prevention of extreme potassium and sodium depletion, by means of intravenous administration of these electrolytes, was associated with the same type of "antiketotic" effect of ACTH and cortisone seen in the nondiabetic subject. D/N ratios in excess of 3.65 were not obtained in this study, probably because of the relatively modest hyperglycemia.

of ACTH and cortisone in this patient was much the same as that obtained in the nondiabetic, in that the blood sugar level rose progressively; and the blood and urinary ketones, after an initial rise, fell to levels similar to those observed prior to ACTH and cortisone administration. D/N values in excess of 3.65 were not obtained in this patient, probably because of the relatively modest degree of hyperglycemia which was produced by hormonal administration.

Study 4. The next study was carried out in a female patient, BROS, aged 33 years, with diabetes of moderate severity (Figs. 4a and b). Throughout the study she was maintained on a quantitatively constant formula diet made up of fat and protein and suitably fortified with minerals and essential vitamins. The diet was administered through a nasogastric tube at hourly intervals throughout the twenty-four hours. "Regular"

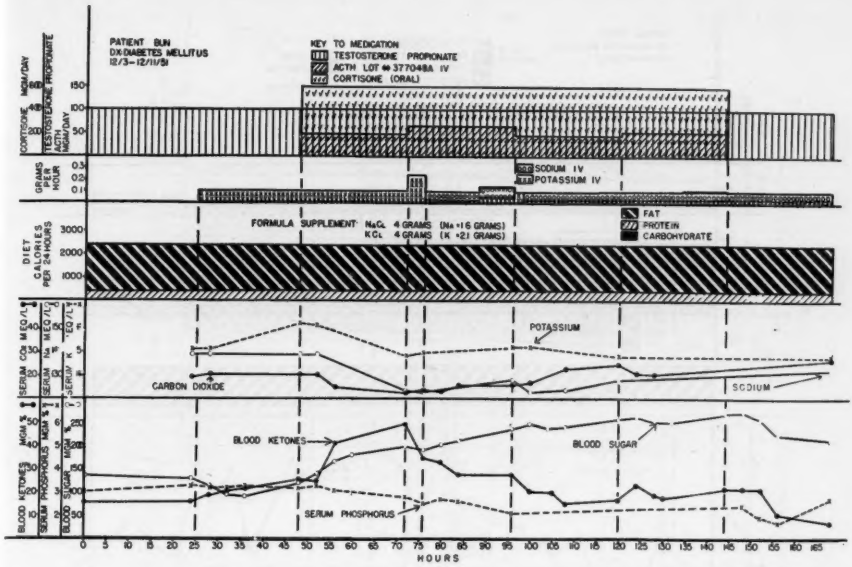

FIG. 3b. The levels of blood sugar, ketones and serum electrolytes in Patient BUN. Despite sodium and potassium supplementation, the patient showed a negative balance for both ions, probably because of the combined effects of the hormones plus the hyperketonemia.

insulin was administered every six hours in the total amounts indicated. Additional amounts of potassium and sodium were administered intravenously, as indicated. After a brief control period, 600 mg. of hydrocortisone acetate and subsequently 600 mg. of cortisone acetate were administered at the rate of 25 mg. every hour through the nasogastric tube. An insulin tolerance test performed after four days' administration of steroid showed fair insulin sensitivity. On the sixth day of steroid administration, some decrease in insulin response was noted. There was progressive elevation of the blood sugar level during the period of steroid hormone administration, and a progressive but not precipitous rise in blood ketones. D/N ratios of 4.9, 5.3, 4.8 and 4.5, respectively, were obtained during four days of the study. Even though large amounts of potassium were administered, the patient showed a negative potassium balance throughout. Despite steroid hormone administration, and presumably because of the hyperketonemia, there was also a net loss of sodium.

From the foregoing and other short-term studies not included here, it was felt that the observations up to this point were compatible with the concepts previously advanced (see preceding sections) to explain the differ-

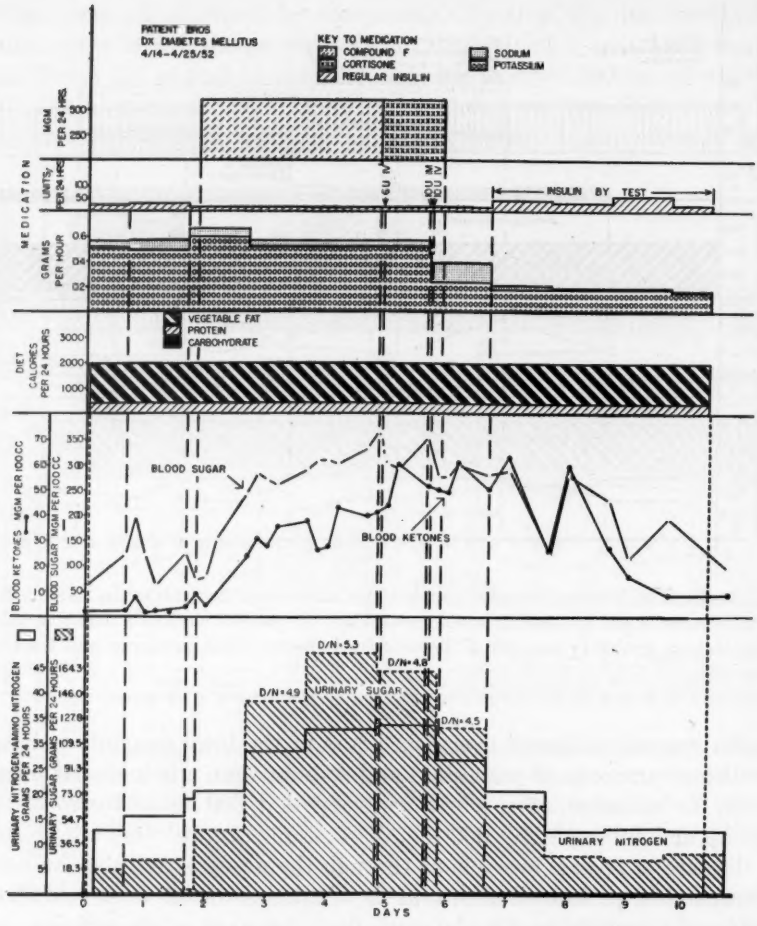

FIG. 4a. Administration of Compounds F and E to diabetic Patient BROS resulted in D/N ratios well in excess of 3.65. Potassium and sodium supplementation appeared to prevent major insulin resistance.

ent responses obtained in diabetics receiving ACTH and cortisone as compared to nondiabetics; further, they were compatible with the concept that potassium and/or sodium depletion per se was associated with the development of insulin resistance, and that potassium and/or sodium supplementation tended to prevent the development of insulin resistance; and finally, they were compatible with the concept that a portion of the diabetogenic effect of adrenal steroids could be attributable to acceleration of neoglucogenesis from fat (see particularly the studies in Patient BROS).

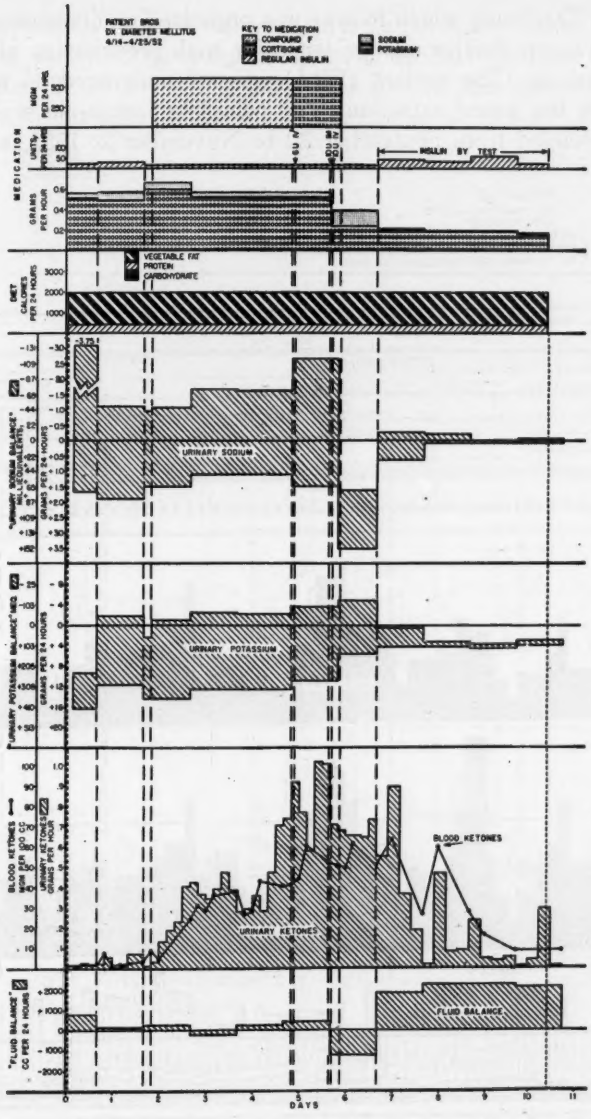

FIG. 4b. Despite sodium and potassium supplementation, Patient BROS showed a negative sodium and potassium balance, probably because of the dual effect of the hormones plus the hyperketonemia.

Study 5. The study which follows was organized and carried out specifically to evaluate further the evidence for such acceleration of neoglucogenesis from fat. The patient (FRA) was a female aged 35 with severe diabetes of ten years' duration. The complete metabolic study in this patient extended from September 30 to November 2, 1952, and will be

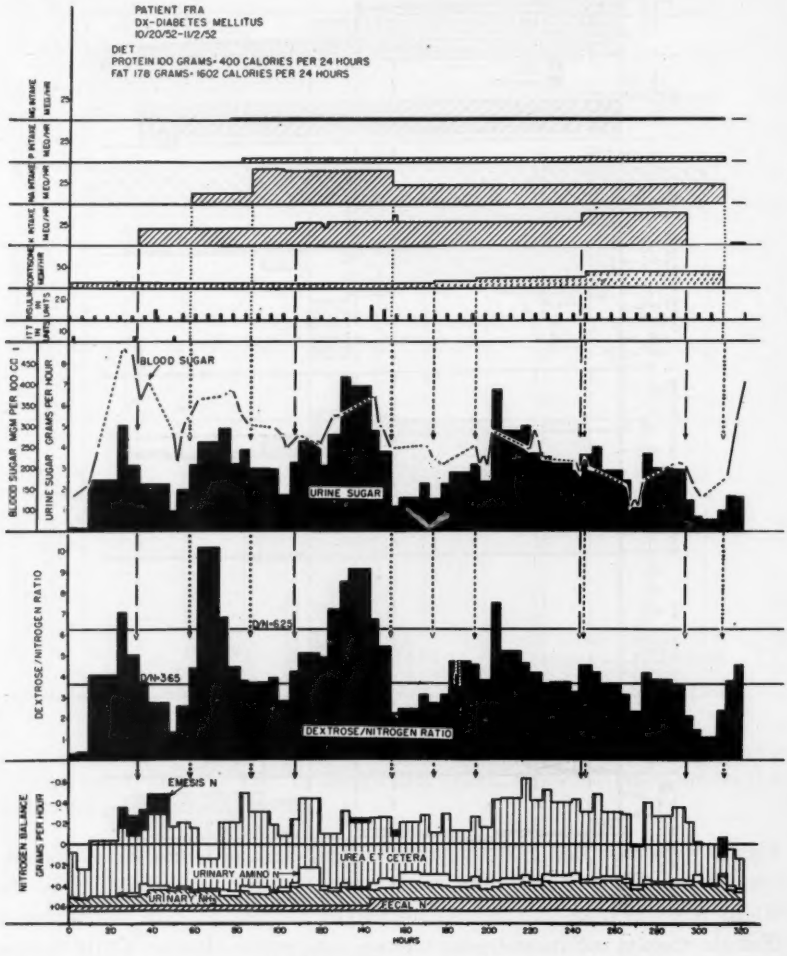

FIG. 5a. Studies in a case of severe diabetes (Patient FRA). Despite large amounts of cortisone, there was no development of insulin resistance; on the contrary, the blood sugar level fell progressively. D/N ratios well in excess of 6.25 were obtained during significant portions of the study. The patient showed a negative nitrogen balance throughout.

reported separately. Herein will be included a portion of the data obtained through the period of most intensive hormonal therapy from October 20 to November 2, 1952. During this entire period, the patient was maintained on a formula diet containing 100 grams of protein and 178 grams of

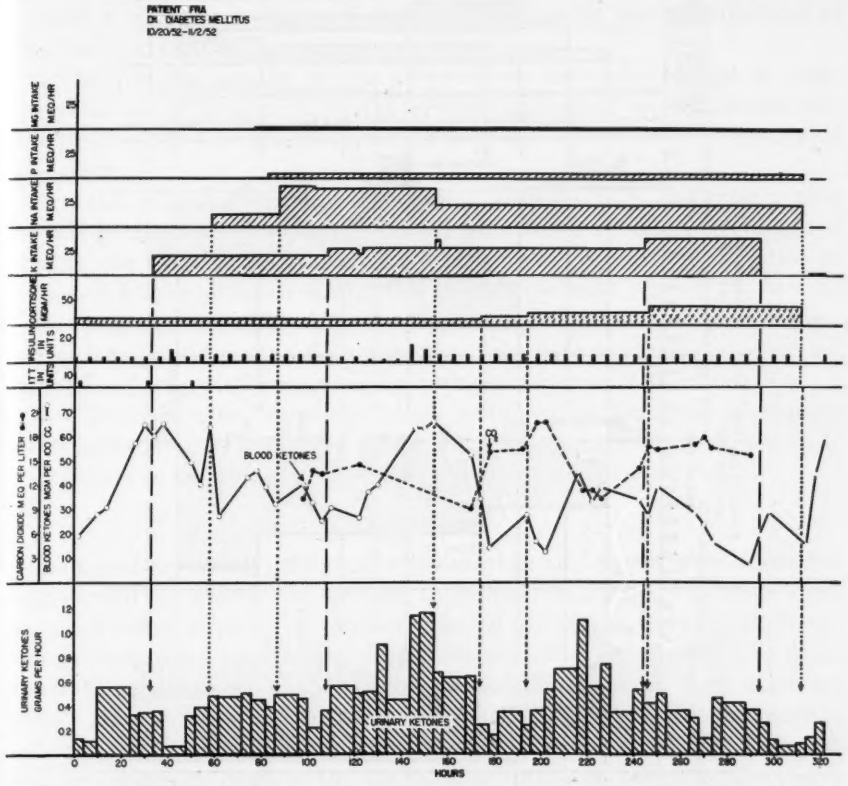

FIG. 5b. Major hyperketonemia appeared only when insulin administration was diminished. Essentially, cortisone appeared to produce the same effect upon ketones in this patient as in the nondiabetic subject.

fat, supplemented with relatively large amounts of essential vitamins, with basic minerals, and with additions of potassium and of sodium at specific times as shown in Figures 5a to c. Throughout this portion of the study, the patient showed negative nitrogen, magnesium and sulfur balances. Urinary sodium and potassium were determined at four-hourly intervals during most of the study, and sufficient potassium and sodium were added to the diet to maintain equilibrium. In the final portion of the study, it was necessary to administer 38.73 milliequivalents of potassium per

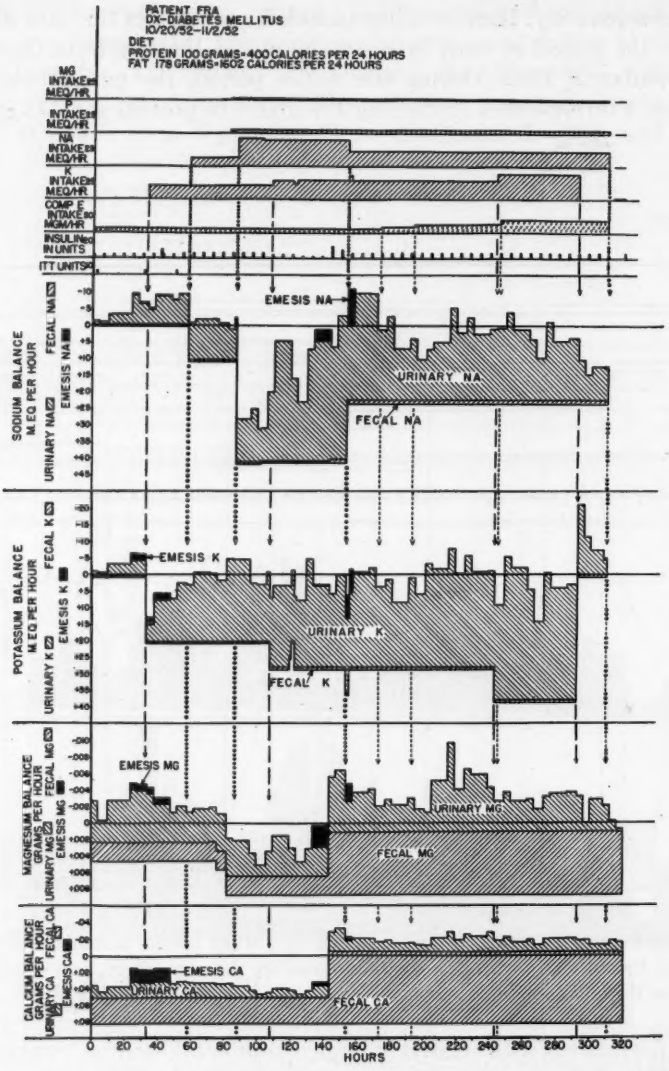

FIG. 5c. Electrolyte balances in Patient FRA. Relatively huge amounts of potassium were required to maintain a positive potassium balance in the presence of a dosage of 1,200 mg. of cortisone daily.

hour in order to maintain a positive potassium balance (69.4 grams of potassium chloride daily). Under these experimental conditions, the following observations were made:

1. No "insulin resistance" was observed at any time during the course of the study. During the second half of that portion of the study here discussed, a constant dosage of 6 units of plain insulin was administered at six-hourly intervals.

2. Despite (or because of) the progressively increasing dosage of cortisone and a constantly high level of protein catabolism, after an initial rise, the trend of the blood sugar level was progressively downward throughout the study.

3. D/N ratios in excess of 3.65 were obtained during the greater part of this portion of the study; D/N ratios in excess of 6.25 were obtained during one 24-hour period and for other shorter periods; D/N ratios in excess of 9 were obtained during two 10-hour periods.

4. As in the case of the blood sugar, the blood and the urinary ketones, excepting specific peaks relating for the most part to significant decrease in insulin dosage, fell progressively during the course of the study.

5. The respiratory quotient values obtained at the time of highest D/N ratios were as low as .34. Those obtained during periods of low D/N ratios were in the expected "normal" range for this type of diet.

DISCUSSION

As noted previously, the studies included in this report were organized and carried out around the working hypothesis that adrenal steroids exert an accelerating effect upon the formation of carbohydrate from fat. In order to document such an hypothesis unequivocally, it is essential to demonstrate in the urine of diabetic or nondiabetic subjects, maintained on diets entirely free of carbohydrate, amounts of carbohydrate significantly in excess of those which can be obtained from catabolized endogenous and/or exogenous protein. On the basis of studies in the older literature, carried out chiefly in phloridzinized animals, many investigators concluded that approximately 58 per cent of catabolized protein could be transformed into carbohydrate, *i.e.*, urinary nitrogen multiplied by a factor of 3.65 would represent the maximal urinary carbohydrate to be obtained from such catabolized protein. Recent studies have suggested that considerably more than 58 per cent of protein can be transformed into carbohydrate (7). For this reason we felt that, in view of the controversial nature of the subject, it would be well to set a goal of a D/N ratio for significant periods of time in excess of 6.25, *i.e.*, in excess of the amount of urinary carbohydrate which could be obtained if *all* of the catabolized protein were metab-

olized to carbohydrate. In addition to this, the total urinary carbohydrate should be corrected for the amount which could be formed from the glycerol moiety of catabolized fatty acids. To know the maximal amount of carbohydrate from this source, it would be necessary to know the total catabolism of fat at any given time. Such a figure may be estimated on the basis of average oxygen consumption in the course of a given study.

Because of our early difficulties with the development of insulin resistance, studies were carried out with great caution. As a result, D/N ratios in excess of 6.25 observed for significant periods of time were not obtained until the most recent study in patient FRA. If the D/N ratios obtained in this patient are corrected for the maximal amount of carbohydrate which could be derived at any period from the glycerol of catabolized neutral fat, a comfortable margin of urinary carbohydrate still is present, which must be derived from some source other than either catabolized protein or glycerol. In a patient maintained for a prolonged period of time on a completely carbohydrate-free diet, the only remaining source of such carbohydrate would appear to be fatty acid.

One loophole remains to be closed, namely, the possibility that over a period of a few hours significant amounts of urinary carbohydrate could be attributable to stored hepatic glycogen. The magnitude of the findings in Patient FRA make this improbable, but not impossible. In the light of knowledge thus far obtained, it is planned to carry out further studies in diabetics, the objective being that of maintenance of sufficiently high blood sugar levels for sufficiently long periods of time to exclude in a quantitative fashion any possibility that stored glycogen could result in a spuriously high D/N ratio. The major practical difficulty with this procedure lies in the disturbed metabolism attendant upon prolonged hyperglycemia and the excessive protein catabolism which occurs under these conditions. With adequate fluid and electrolyte replacement and with protein ingestion sufficient to prevent negative protein balance, however, these problems can be minimized.

The relationship of specific electrolytes to carbohydrate metabolism is discussed in detail elsewhere (2). It may not be amiss at this time, however, to emphasize again that prevention of depletion of potassium and of sodium was associated with complete absence of insulin resistance. McQuarrie *et al.* (8) noted modification of nonsteroid diabetes by sodium some years ago.

One may also speculate upon the interpretation of the unorthodox observation that rather prolonged administration of large amounts of 11-dehydro-17-hydroxycorticosterone to a patient with severe diabetes under suitable conditions resulted in a progressive *fall* rather than a rise in the level of blood sugar (insulin administration remaining constant). Inhibi-

tion of pituitary diabetogenic factor(s) by such steroid administration might explain such an observation.

It is of clinical interest that no diabetic patient who received ACTH or cortisone had a permanent increase in the severity of the diabetic state after cessation of hormone administration. In fact, in 2 instances the diabetes became less severe in terms of insulin requirement. This does not imply a cause-and-effect relationship. It is possible that in insulin-resistant diabetes, when the resistance is allergic in nature, ACTH and cortisone may have clinical usefulness.

SUMMARY

Studies carried out in this laboratory over a period of nearly five years are compatible with the concept that cortical steroids accelerate the formation of carbohydrate from fat. They indicate further that prevention of depletion of potassium, and possibly of sodium, may be of importance in preventing steroid hormone-induced insulin resistance.

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THE PREDIABETIC SYNDROME. LARGE BABIES AND THE (PRE)DIABETIC FATHER*

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PREDIABETIC AND (PRE)ACROMEGALIC WOMEN

MANY women destined to have diabetes divulge their fate by producing infants which are large (1–10), or dead (1–5, 11–16).

The high rates of fetal loss and the production of large babies are exemplified by the pregnancies of prediabetic women in the Cape Town region of South Africa. The methods used for collecting the data and the detailed results of this study have been described elsewhere (5). Suffice it to say that 62 per cent of women in the years before they became diabetic claimed to have produced at least one child weighing over 10 pounds, compared with only 12 per cent of normal control mothers. This is further shown in Table 1, in which the first and last horizontal rows contrast the babies of prediabetic with those of control mothers. The much greater incidence of large babies and of late fetal death in the prediabetics' babies leads us in the Cape to suspect diabetes strongly from such an obstetrical history.

The work of Young (17) has established the probable identity of the diabetogenic and growth factors of the anterior pituitary. Long-standing overproduction of this hormone has been suggested to account both for the large babies and for the later development of maternal diabetes (3, 10, 14). The high fetal death rate also has been attributed to the same overproduction, or perhaps to its extension to include excessive production of gonadotropins (18). However, this interesting armchair theory has no factual support. As pointed out by Wilder (19), the usual features of hypophysial overactivity do not appear in these mothers, and acromegaly

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TABLE 1. CHILDREN OF (PRE)DIABETIC FATHERS, AND PREDIABETIC, (PRE)ACROMEGALIC AND CONTROL MOTHERS

Children	Total No. of children	Per cent				
		Under 8 lb.	8–8.9 lb.	9–10 lb.	Over 10 lb.	Stillborn
Of prediabetic mothers	428	22	32	15	31	15
Of (pre)diabetic fathers	398	48	32	9.3	10.7	<3
Of (pre)acromegalic* mothers	35	54	26	8.5	11.5	0
Of control mothers	819	72	19	5.3	3.7	<3

* The term "(pre)acromegalic" includes both mothers who were acromegalic when pregnant and those in whom this disease developed later.

Only 1 mother out of 8 in whom acromegaly actually developed during pregnancy had a child weighing over 9 pounds at birth.

itself is seldom associated with clinical diabetes. Moreover, an excess of circulating growth hormone has not been demonstrated in diabetes at any stage. Barns and Swyer (20) injected pregnant rats with purified active growth hormone but were unable to demonstrate any definite effect on the fetal weights. If growth hormone were indeed the cause of diabetic macroinfantia, then the babies of acromegalic women would presumably be exceptionally large. We believe that acromegaly is accompanied by an excess of circulating growth hormone, and Kinsell (21) has demonstrated this substance in the blood stream in one case. It is therefore of interest to investigate the birthweights and fetal loss rate in the infants of acromegalic mothers.

The distribution of birthweights in 35 babies born to 11 acromegalic mothers is shown in Table 1. From this small sample it appears that the birthweights tend to be above the control figures, but they are not as large as those for prediabetics' babies. Among 8 women in whom acromegaly probably began or was actively progressing during pregnancy, 1 alone produced a baby over 9 pounds in weight. There were no stillbirths or early neonatal deaths in these pregnancies.

THE (PRE)DIABETIC FATHER

We must, then, look elsewhere for the explanation of the prediabetic syndrome. In the past, it has been tacitly assumed that the mother is wholly responsible for the condition of the child. It seemed to us possible that the mother's internal environment might not be the sole factor causing macroinfantia, but that the father might also have something to do with the condition of the child, and that the high birthweight might be an inherited characteristic linked in some way with the diabetic genetic make-

TABLE 2. CHILDREN OF (PRE)DIABETIC AND CONTROL FATHERS
(Cape Town data)

Children	Total No. of children	Per cent				
		Under 8 lb.	8–8.9 lb.	9–10 lb.	Over 10 lb.	Stillborn
Of (pre)diabetic fathers	235	40	36	11	13	3
Of control fathers	147	60	32.6	2	5.4	3

Chi square (χ^2) = 25.9
N = 3
P = .000012

up. In this connection the existence of familial *nondiabetic* macroinfantia is well attested (8, 22, 23).

In this report are presented two series of birthweights of infants of diabetic and prediabetic fathers, one from Cape Town and one from Boston.[1] The inherent inaccuracies in the use of direct questioning of known diabetics (as has been used here) have been previously discussed (5). On the other hand, the large nondiabetic control series drawn from the same type of people appear to offer a reasonable basis for comparison. Incidentally, whether the father is diabetic or prediabetic at the time of conception makes no difference to his possible contribution to fetal weight, since we are interested in his germ plasm and not in his internal environment. Fathers therefore are included as "(pre)diabetic," whether truly prediabetic or actually diabetic at the critical time.

TABLE 3. CHILDREN OF (PRE)DIABETIC AND CONTROL FATHERS
(Boston data)

Children	Total No. of children	Per cent			
		Under 8 lb.	8–8.9 lb.	9–10 lb.	Over 10 lb.
Of (pre)diabetic fathers	163	59	27	6.6	7.4
Of control fathers	249	76.2	15	5.6	3.2

Chi square (χ^2) = 15.5
N = 3
P = .0012

[1] Sincere thanks for cooperation in obtaining the latter series are due to Dr. Priscilla White, Dr. Reed Harwood and Dr. David Hurwitz, from the Joslin Clinic, the Massachusetts General Hospital and the Boston City Hospital, respectively.

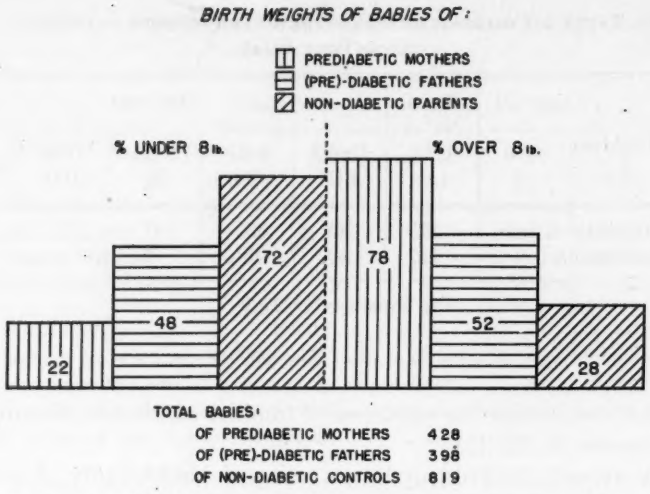

FIG. 1. Proportion of children weighing under 8 pounds and over 8 pounds at birth in the three groups designated shows the intermediate position of babies of (pre)diabetic fathers between those of prediabetic mothers and of normal parents.

The results are shown in Tables 1, 2, and 3, from which it may be seen that there is not only a large proportion of 10-pound babies in the (pre)diabetic-father groups, but there is a general tendency for their babies to be larger than those of the respective control groups. The incidence of large babies is not as marked, however, as in the prediabetic-mother groups, and lies somewhere between the incidence in these women and in their controls; Table 1, which summarizes the complete data, shows the intermediate position—somewhat similar to that of the (pre)acromegalic mothers' group. Figure 1 also demonstrates the intermediate position of

TABLE 4. (PRE)DIABETIC FATHERS AND CONTROL FATHERS
(Combined Cape Town and Boston data)

	Total No.	Per cent	
		Fathers with at least one child over 9 lb.	Fathers with at least one child over 10 lb.
(Pre)diabetic fathers	117	44	20
Control fathers	151	14	8
Difference	—	30	12
S.E. of difference	—	9.8	4.3
Difference/its S.E.	—	3.0	2.8

(pre)diabetic fathers' infants, by comparing the percentages of babies weighing over 8 pounds in each group. Further, Table 4 shows that 20 per cent of all the diabetic fathers claimed infants weighing over 10 pounds at birth, compared with only 8 per cent of controls.

In each series, the difference between the (pre)diabetic-father group and the control group is highly significant when analyzed by the chi-square method. There is less than one chance in a million that the differences found could have arisen fortuitously.

In contradistinction to the babies' size, the stillbirth rate was found to be no greater in children of (pre)diabetic fathers than in children of the controls (Tables 1 and 2).

INTERPRETATION

The evidence suggests that the tendency to produce large babies is *partly* an inherited characteristic combined with a tendency to diabetes, passed on by the male as well as the female, and *partly* an effect of maternal internal environment.

Figure 2 attempts to show the relative importance of the maternal internal environment and of inheritance, based on the Cape Town figures, referring only to percentages of infants with birthweights over 10 pounds. The difference between the figures for prediabetic mothers and (pre)-diabetic fathers must be due to purely maternal factors; the difference

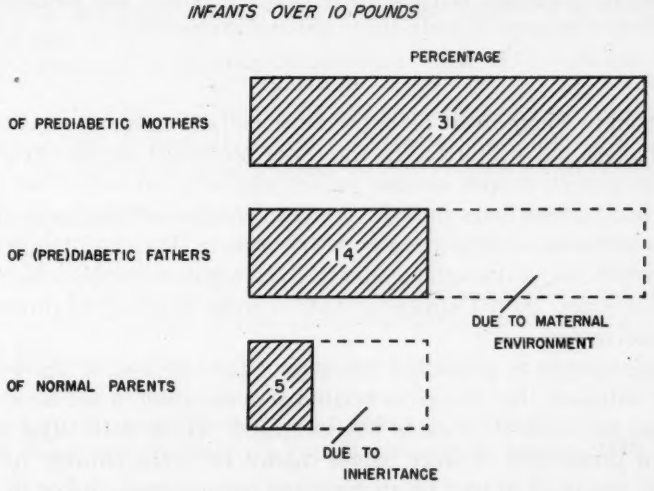

FIG. 2. Comparison of proportion of infants over 10 pounds born to prediabetic mothers, to (pre)diabetic fathers, and to normal parents. Blocked differences show, respectively, the apparent importance of maternal internal environment and of inheritance in producing large babies.

between the figures for (pre)diabetic fathers and controls must be due to inherited factors.

On the other hand, since the late fetal death rate in the (pre)diabetic-father group and in the controls is virtually the same, the excessive fetal loss in the prediabetic-mother group must be entirely mediated by special maternal factors (Fig. 3).

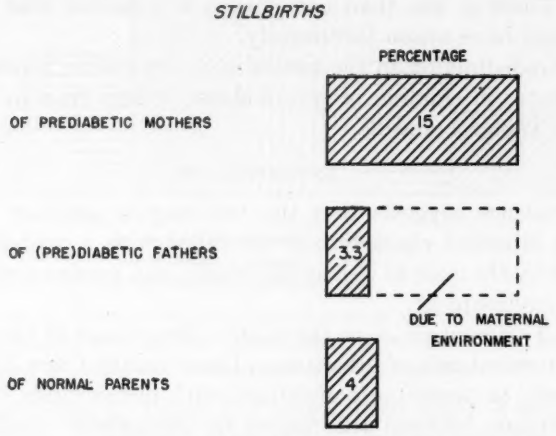

FIG. 3. Comparison (in a manner similar to that in Figure 2) of the proportion of stillbirths for prediabetic mothers, for (pre)diabetic fathers, and for normal parents. The difference seems to be solely due to maternal environment.

SUMMARY

Women in whom overt diabetes develops later in life are apt to produce large babies and stillbirths. In the series presented in this report over 60 per cent of such women claimed babies who weighed more than 10 pounds at birth. At present, the most favored explanation attributes this phenomenon to an excess of circulating growth hormone. There is little evidence for this hypothesis, and much against it. Acromegalics, for instance (in a small personal series) do not appear to have a great tendency to produce babies of excessive size.

New evidence is presented concerning the children of diabetic fathers which indicates that the birthweights of such children are above average, whereas the stillbirth rate is not increased. These data suggest that the onus of production of large babies cannot be borne entirely by maternal factors, but must in part be an inherited phenomenon, linked in some way to the diabetic genetic constitution of either parent. The concept of the "prediabetic father" is thus born.

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EFFECT OF ALPHA-CELL DESTRUCTION ON THE HGF CONTENT OF THE CANINE PANCREAS*†

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IT APPEARS to be an established fact that the pancreas produces, in addition to insulin, another substance which influences the blood sugar level. This substance is present in most insulin preparations and causes an initial transitory hyperglycemic effect when injected intravenously. Originally believed to be a contaminant of insulin (1–7), it has been variously termed hyperglycemic glycogenolytic factor (HGF) or glucagon. In more recent years it has been shown to be a distinct entity. Since the substance has been isolated from the normal and alloxanized pancreas (8) as well as from the pancreas of alloxan diabetic animals in which the exocrine portion has been fibrosed by duct ligation (9), a number of investigators believe that it is derived from the pancreatic alpha cells. This suggestion is supported by the finding that an extract with similar hyperglycemic properties can be prepared from the upper three-fourths of the gastric mucosa and the duodenum of the dog (8); these areas contain argentaffin cells which are histochemically similar to, but not identical with the alpha cells of the pancreas. Other tissue extracts do not seem to contain this principle.

A physiologic hyperglycemic action of the pancreas has also been inferred from cross-circulation experiments in dogs (10, 11) and from a comparison of the severity of alloxan diabetes, clinical diabetes, and surgical diabetes (12, 13).

Recently it has been reported that cobaltous chloride produces selective damage to the alpha cells of the guinea pig after subcutaneous injection (14, 15). We have demonstrated that alpha-cell damage can also be obtained in rabbits and dogs after the intravenous administration of cobaltous chloride (16, 17, 18). We also reported that the cobalt-induced autolysis of the alpha cells is not accompanied by persistent changes in the blood

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sugar level in either the normal rabbit or dog and that in alloxanized rabbits and dogs the diabetes is not ameliorated (17, 18). Furthermore, the initial short-lasting hyperglycemic reaction which occurs after cobalt administration cannot be ascribed to the alpha-cell damage, since it was demonstrated in depancreatized dogs and in similar animals with the upper gastrointestinal tract also resected (18).

In order to investigate further the relationship between the alpha cells and HGF, we have prepared pancreatic extracts from cobalt-treated animals and have compared the hyperglycemic effect of these extracts with those of untreated animals. The results of these experiments are reported here.

MATERIALS AND METHODS

This study was carried out with pancreatic extracts derived from 20 dogs of either sex, weighing from 15 to 20 kilograms. The animals were divided into two main groups. The first group consisted of 10 normal dogs, 2 of which received 200 mg. of cobaltous chloride intravenously as 1 per cent solution on the day prior to, and on the day of pancreatectomy; 3 received 3 doses of 200 mg. of cobaltous chloride at 1½-hour to 2-hour intervals; pancreatectomy was performed one hour after the third dose. Pancreases from 5 untreated dogs were used as controls.

The second group consisted of 10 dogs made diabetic by the intravenous administration of 75 mg./Kg. of alloxan monohydrate (Eastman) as a 1 per cent solution in water. Three of these 10 dogs were used as controls. Of the remaining 7 animals, 3 received 200 mg. of cobaltous chloride on two successive days and 4 were given 200 mg. for three doses at 1½-hour intervals just prior to pancreatectomy. All pancreases were frozen immediately after extirpation, using carbon dioxide under pressure, and were then placed in a freezing compartment until the extraction could be performed.

The pancreatic extracts were prepared by the method of Best *et al.* (19). The dried extracts were kept in the icebox until ready for use. They were redissolved in physiologic saline (sodium chloride) adjusted to pH 2.4 with HCl; 1 cc. of solvent was used for each gram of pancreas. Then 1 cc. of the extract solution was injected intravenously into a fasting normal rabbit weighing 2 to 4 Kg. Blood samples for glucose determination were withdrawn in duplicate from the ear veins just prior to, and 5, 10, 20, 30, 45 and 60 minutes after the administration of the extract. As controls, fasting normal rabbits were injected with 1 cc. of physiologic saline, acidified to pH 2.4, as well as physiologic saline per se. Extracts of dog spleen prepared similarly to the pancreatic extracts were used for control experiments.

In addition, 2-cc. portions of some of the same pancreatic extracts were injected into fasting normal dogs two hours after the induction of intraperitoneal Nembutal anesthesia, and blood was withdrawn for glucose determination at the same time-intervals as in the rabbit. When necessary, as indicated by a returning corneal reflex, the test animals were given additional small doses of Nembutal. The 2-hour interval after anesthesia was chosen, since it had previously been demonstrated that it allows for recovery of the animal from the initial stress accompanying the induction of anesthesia (20). As a further control, blood samples for glucose determination were withdrawn at 15-minute intervals for one hour prior to the administration of the extracts. Similar experiments were also repeated, using splenic extracts and acidified salt solution instead of the pancreatic

extracts. The blood glucose was determined by the Nelson modification of the Folin-Wu micro method (21).

In order to verify the effects of cobalt and alloxan on the islets, small portions from each pancreas were removed for histologic examination at the time of the pancreatectomy. The sections were stained with haematoxylin and eosin after formalin fixation, as well as by Gomori's chrome-alum-haematoxylin and phloxine technique after Bouin fixation (22).

RESULTS

The mean change in the blood sugar level of the fasting rabbit after the injection of each type of extract, as compared to the controls, is presented in Table 1. In Figure 1 representative curves of individual experiments

TABLE 1. MEAN BLOOD SUGAR RESPONSES OF FASTING NORMAL RABBITS TO THE INTRAVENOUS INJECTION OF PANCREATIC EXTRACTS FROM COBALT-TREATED AND/OR ALLOXANIZED DOGS AND OF EXTRACT OF CANINE SPLENIC TISSUE, PHYSIOLOGIC SALINE AND ACIDIFIED PHYSIOLOGIC SALINE*

Material injected	Time in minutes						
	0†	5	10	20	30	45	60
	Blood sugar, mg./100 cc.						
Extract of pancreas of normal dogs—5‡	64	72	47	20	21	20	25
Extract of pancreas of cobalt-treated dogs—5	89	101	62	43	36	53	60
Extract of pancreas of alloxanized dogs—3	85	108	120	80	75	84	81
Extract of pancreas of alloxanized cobalt-treated dogs—7	79	92	114	92	70	59	71
Extract of canine splenic tissue—3	81	83	77	87	85	84	82
Physiol. saline (NaCl)—5	63	66	58	68	79	69	67
Physiol. saline acidified to pH 2.4 with HCl—5	89	92	95	89	91	93	94

* Extracts prepared by the method of Best, Haist and Ridout (19).

† Test material was injected immediately after withdrawal of the fasting blood sample.

‡ The numeral indicates the number of extracts used.

are demonstrated. As was to be expected, extracts from normal dog pancreas usually elicited a short-lasting initial hyperglycemia with the peak at five minutes and a subsequent decline to hypoglycemic levels, due to the insulin content. In occasional experiments the initial hyperglycemia was not observed, even though in another trial the same extract elicited a hyperglycemic reaction. This discrepancy is interpreted as being due either to a depletion of carbohydrate stores in some of the test rabbits or to an increased sensitivity of some rabbits to the action of insulin, which masks the initial hyperglycemic effect of the extract.

The extracts from cobaltized pancreas in which the alpha cells were histologically markedly damaged or had disappeared completely (Fig. 2b), when injected into rabbits gave typical biphasic curves. These curves did not differ significantly from the effect of extracts from normal pancreas (Table 1 and Fig. 1).

Extracts from alloxanized pancreas in which there was marked beta-cell destruction but in which the alpha cells were intact (Fig. 2c), caused a greater and more prolonged hyperglycemia than extracts from normal

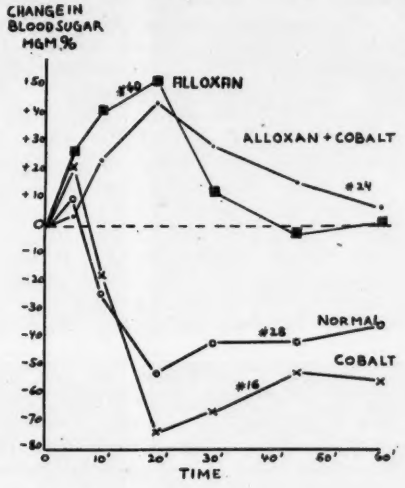

FIG. 1. Typical blood sugar curves in fasting rabbits after the intravenous injection of pancreatic extracts from normal, alloxanized, cobalt-treated and alloxanized cobalt-treated dogs.

pancreas. After the injection of these extracts the peak blood sugar level was usually reached at ten to twenty minutes. In some instances there was a mild secondary depression of the blood sugar concentration but in most cases no insulin effect was observed. A typical curve is depicted in Figure 1. Extracts from the pancreas of alloxan-diabetic dogs treated with cobalt, which histologically showed both marked alpha-cell and beta-cell destruction (Fig. 2d), still contained hyperglycemic principle. The curves obtained were in all respects similar to those from the alloxanized animal (Fig. 1 and Table 1).

Table 2 records the effect of the injection of some of the same extracts into dogs. It can be noted that prior to the administration of the extract the blood sugar level of the test animal, if anything, tended to decline. In all instances a significant transient hyperglycemia occurred, even with

TABLE 2. BLOOD SUGAR RESPONSE OF ANESTHETIZED FASTING NORMAL DOGS TO THE INTRAVENOUS INJECTION OF PANCREATIC EXTRACTS FROM COBALT-TREATED AND/OR ALLOXANIZED DOGS, OF EXTRACT OF CANINE SPLEEN, AND OF ACIDIFIED PHYSIOLOGIC SALINE*

Material injected	Dog	Time in minutes									
		Control period				After administration of extract					
		0†	15	30	45‡	5	10	20	30	45	60
Extract of pancreas of normal dogs	#32	90	95	87	88	97	106	103	73	53	59
	#30	89	80	91	82	122	126	74	49	29	27
Extract of pancreas of cobalt-treated dogs	#41	76	75	81	68	107	102	59	36	25	29
	#12	86	84	92	80	104	124	94	51	26	35
Extract of pancreas of alloxanized dogs	#40	91	82	72	76	122	132	105	86	80	84
	#18	77	74	65	67	93	102	62	47	51	71
Extract of pancreas of alloxanized cobalt-treated dogs	#24	88	76	72	82	92	126	126	100	76	80
	#34	93	88	82	72	103	130	105	95	90	80
	#36	93	90	78	84	117	121	76	51	39	58
Extract of splenic tissue		82	84	84	80	82	80	80	82	86	84
		99	86	82	86	94	94	97	84	86	84
Acidified saline (NaCl)		64	62	69	64	60	58	62	60	63	63
		86	82	79	78	71	75	77	85	84	88

* Extracts were prepared by the method of Best, Haist and Ridout (19).

Dry extract equivalent to 2 Gm. of the original tissue was dissolved in 2 cc. of acidified saline and injected.

† First control sample withdrawn one hour after the intraperitoneal administration of 30 mg./Kg. of Sodium Nembutal.

‡ Test material was administered intravenously immediately after withdrawal of this blood sample.

extracts from those pancreases in which most alpha cells were completely destroyed. On the other hand, insignificant blood sugar changes accompanied the injection of acidified saline or splenic extracts (Table 2).

DISCUSSION

Our results demonstrate the presence of a hyperglycemic principle in the extracts from pancreatic glands, when the alpha cells have been damaged or destroyed by cobalt. The hyperglycemic effect of these extracts was similar to that of extracts from normal glands. This makes it improbable that the alpha cells are the site of origin of this substance. Two objections might be raised to this conclusion: Firstly, the elevation in the blood sugar level of the rabbits or dogs after the administration of the extracts is a qualitative and not a quantitative measure of HGF content. Secondly,

FIG. 2. **a)** Pancreatic islet of normal dog. Arrows indicate alpha cells. Gomori stain. ×450. **b)** Dog pancreatic islet composed of beta cells only. Biopsy specimen obtained four hours after the injection of cobalt. Gomori stain. ×450. **c)** Pancreatic islet of alloxanized dog, composed of alpha cells only. Arrows indicate empty areas after autolysis of beta cells. Gomori stain. ×450. **d)** Pancreatic islet of alloxanized cobalt-treated dog. There is almost complete destruction of alpha and beta cells. Arrow indicates a degenerated alpha-cell remnant. Gomori stain. ×450.

even in a pancreas showing the most marked alpha-cell destruction there were still occasional islets in which morphologically intact alpha cells were observed. These objections seem to be obviated by the fact that the blood sugar-raising potency of the extracts was of similar degree, regardless of whether the extracts were from normal pancreatic tissue or from organs showing various degrees of alpha-cell destruction. Furthermore, even when histologic examination proved that there was almost complete loss of both alpha and beta cells after cobalt and alloxan administration, apparently normal quantities of blood sugar-raising principle could be demonstrated (Fig. 1, dog 24).

Vuylsteke *et al.* (23) extracted guinea-pig pancreas after subcutaneous cobalt administration and found a decrease in the HGF content, which they considered to be significant. Important as this observation is, it must be emphasized that their extracts still exhibited marked hyperglycemic activity. One must question the validity of the quantitative assay of their extracts in view of the difficulties inherent in working with organs of such small weight. The species difference also may be of importance. In this connection it is noteworthy that although hyperglycemic principle is obtained from the gastric mucosa of the dog, no such material is found in the gastric mucosa of the hog (24). This is so, despite the fact that in the hog as in the dog, argentaffin cells similar to the alpha cells of the pancreas are also present in the stomach.

In previous studies it was shown that destruction of the alpha cells by cobalt administration does not lead to hypoglycemia in the normal rabbit or dog. Furthermore, the diabetes of alloxanized rabbits or dogs was not ameliorated by the cobalt administration (17, 18). This evidence was considered to signify that the alpha cells do not play a role in blood sugar homeostasis. The data presented in this study indicate, furthermore, that the hyperglycemic principle does not originate from the alpha cells. In addition, it should be stated that the experiments presented have dealt only with the origin of HGF; they do not imply any conclusions as to the physiologic significance of this substance.

SUMMARY

Extracts from normal and alloxanized pancreas when injected intravenously into fasting rabbits or dogs cause a significant transitory hyperglycemia. Similar results have been obtained after the injection of extracts from pancreas in which the alpha cells have been severely damaged or destroyed by the prior administration of cobalt. These results seem to suggest that the hyperglycemic glycogenolytic factor is not produced by the alpha cells but elsewhere in the pancreas.

Acknowledgment

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AN UNDIAGNOSED ENDOCRINOMETABOLIC SYNDROME: REPORT OF 2 CASES*

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WE HAVE observed 2 cases of a syndrome which does not seem to fit into any of the known clinical pictures.

The main features of this syndrome are an acromegaloid gigantism, hepatosplenomegaly, fatty infiltration of the liver, hyperlipemia, hyperproteinemia, and disturbed carbohydrate metabolism.

CASE REPORTS

Case 1. M.C., a 6-year-old white boy (Figs. 1, 2, 3, 4 and 5—A) (Fig. 6), was seen first in January 1952 and again in June 1952.

The outstanding feature was a marked hepatosplenomegaly, first noticed by the family physician when the boy was 2½ years old. Previous development apparently had been normal, but at that time he had appeared for treatment of a month-long diarrhea and great prostration. During the next six months, his appetite had been excellent, perhaps even exaggerated, and there was no polydipsia. Since then, the patient had been in relatively good health, except for the abdominal protrusion, large joints, and the presence of evening pyrexia.

The only fact of interest in the family history was the death of his oldest sister, at the age of four months. She had had icterus and had seemed to be in pain.

Physical examination: The patient's general appearance was that of a boy from 9 to 11 years old. His height was 123 cm., and his weight was 28.7 Kg. He showed marked abdominal protrusion, muscular hypertrophy, and a pigmentation which gave the impression of being "dirty." A dolicocephalic type with a tendency to oxycephalia, he had projecting superciliary arches and a narrow forehead. Eyegrounds were normal, with no lipemia retinalis. He had an S-shaped pharyngeal vault, and a serrulated border on his inferior central incisors.

The abdomen was large and tense with a projecting navel, and there was a marked venous network on the lateral aspects. The liver was palpable, hard and somewhat tender, with small rounded knobs on the irregular surface. The hard, thin irregular border was about 12 cm. under the right costal margin (hemiclavicular line). The spleen was palpable 15 cm. under the costal margin on the left anterior axillary line, with the greater

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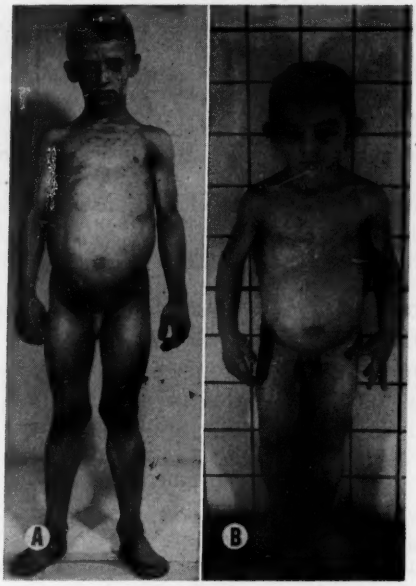

FIG. 1. **A.** *Case 1.* Boy 6 years old; bone age 9 years. Height 125 cm., and weight 30.3 Kg. (normal in Brazil for his age, 109 cm. and 19.8 Kg., respectively). **B.** *Case 2.* Boy 2 years old; bone age 6 years. Height 94.5 cm. and weight 15.7 Kg. (normal in Brazil for his age, 84 cm. and 12.8 Kg., respectively).

axis toward the lower left quadrant. The left submaxillary, retromaxillary, and carotid lymph nodes were palpable, small, movable, hard and not tender. The patient had well developed genitalia corresponding to those of boys several years older. Spina bifida and a coccygeal dimple also were present.

Special mention should be made of the advanced muscular development of the limbs. The muscles were bulky, firm, and well shaped, with turgid, superficial veins. The boy had large hands, feet, and joints (especially the knees). His limbs were covered with fine hair, which also grew on the neck, thorax and pubis. Axillary temperature was 37.4°C. His mental age corresponded to his actual age.

By June 1952[1] his height was 125 cm.; vertex-pubis measurement, 67 cm.; and weight, 30.3 Kg.

Laboratory findings (January 1952): The blood serum had a milky appearance in all tests. Hemogram: red cells 3,800,000/cu. mm.; hemoglobin 11.5 Gm. (76 per cent); hemotocrit 35 per cent; white cells 8,600/cu. mm.; eosinophils 10 per cent, stabs 2 per cent, segmented neutrophils 45 per cent, lymphocytes 38 per cent, monocytes 5 per cent; platelets: 350,000/cu. mm.

The myelogram (sternal puncture) presented a normal appearance. Serologic tests for syphilis gave negative results. The sedimentation rate was 22 mm. the first hour, and 62 mm. the second hour.

[1] See Addenda.

Assay of urinary 17-ketosteroids as dehydroisoandrosterone acetate gave 4.0 mg./twenty-four hours. The plasma cholesterol level was 160 mg. per 100 cc.

In June 1952, blood chemistry showed (all values, per 100 cc. of plasma): protein 8.2 Gm., albumin 4.0 Gm., globulin 4.2 Gm., albumin-globulin ratio 0.9; total lipids 1.2 Gm., cholesterol 166 mg., calcium 10.5 mg., inorganic phosphorus 3.0 mg., alkaline phosphatase 3.2 Bodansky units, sodium 306 mg., and potassium 19 mg.

In the glucose tolerance test, the fasting blood sugar level was 160 mg. per 100 cc. In 10, 30, 60, 90 and 120 minutes after ingestion of 50 Gm. of glucose, it was, respectively, 166, 166, 190, and 237 mg. per 100 cc. At the end of this test there was a glycosuria of 10 Gm.

Liver function tests revealed global hepatic insufficiency.

The urinary excretion of 17-ketosteroids as dehydroisoandrosterone acetate was 2.8 mg./twenty-four hours.

An x-ray study of the skeleton revealed a bone age of 9 years, spina bifida at the level of the first three sacral vertebrae, and a normal sella turcica.

Histopathology of the liver: In all the slides examined, the hepatic parenchyma was represented by a maximum of four to five islets completely surrounded by connective tissue in which reticulin fibers predominated (Fig. 7A). Cells in different stages of vacuoli-

FIG. 2. **A.** *Case 1.* **B.** *Case 2.* Notwithstanding the great resemblance, these patients were not relatives, and were from completely different regions.

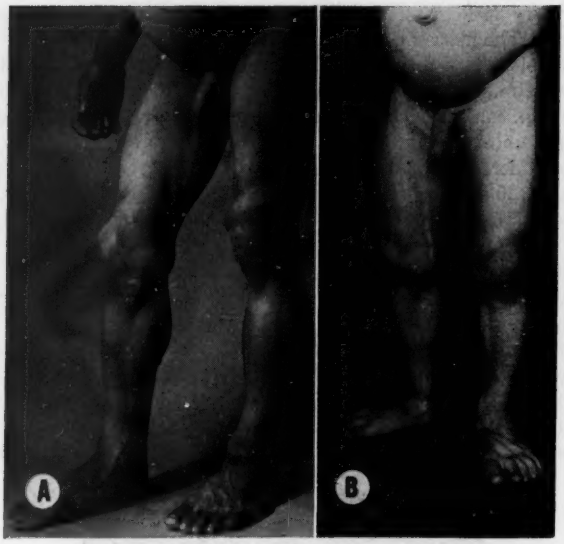

FIG. 3. **A.** *Case 1.* **B.** *Case 2.* Note the extraordinary likeness in the inferior parts of the body. Observe also the phlebomegaly.

zation typified the remaining liver parenchyma. The McManus-Hotchkiss method made it possible to visualize a uniform and intense distribution of glycogen in those hepatic cells free from vacuoles, or those having a small number and of a smaller size (Fig. 8). There was a complete absence of inflammatory infiltrates of any kind. The content of the vacuoles was colored intensely by Sudan IV and became pink with Nile Blue sulphate,

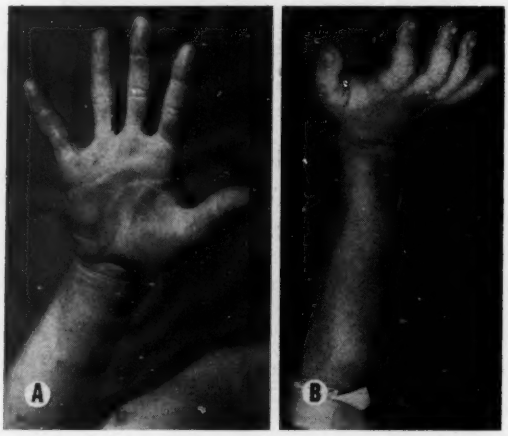

FIG. 4. **A.** *Case 1.* **B.** *Case 2.* Chiromegaly in both patients. Hyperkeratosis also in Case 1.

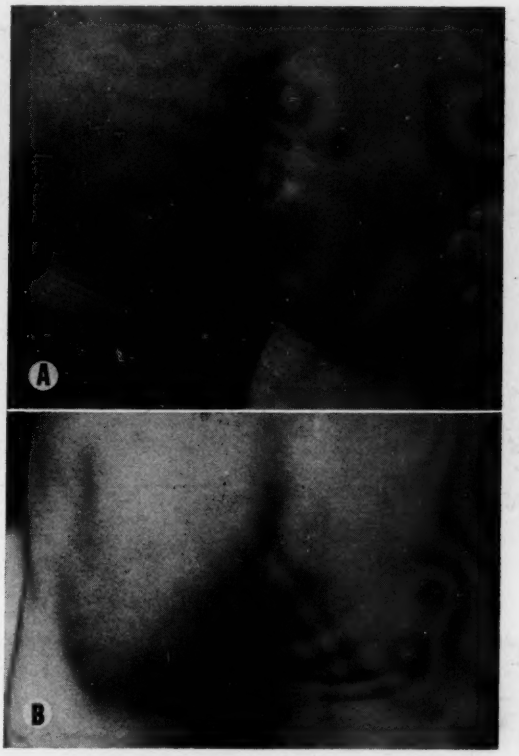

FIG. 5. **A.** *Case 1.* **B.** *Case 2.* Coccygeal dimple in both.

which allowed the existing lipid material to appear as a triglyceride (Fig. 7). The diagnosis was disseminated hepatic fibrosis, with an infiltration of the parenchyma by a lipid substance, a nonsaturated glycerid.

Histopathology of muscles and skin: Bundles of muscle fibers were dissociated by thick sections of adipose connective tissue and bundles of intersecting and coarse elastic fibers (Figs. 9 and 10).

Case 2. J.B.S.G., (Figs. 1, 2, 3, 4 and 5—B) a white boy 2 years and 2 months old, born in Ceara, was first seen in January 1953.

The mother had a normal pregnancy and labor, and at birth the boy was very thin and long. He vomited often during the first month of life. He also had frequent diarrhea, apparently without much colic, which improved with the administration of sulfa drugs. Streptomycin was given on one occasion, at the age of 4 months, during a more severe attack. At the age of 6 months he was examined by a pediatrician, who found large genitalia, and suggested hypophyseal involvement. The patient showed marked muscular and physical development from this age on, although teething and walking occurred at the customary time. When he was 9 months of age, another physician found the liver and spleen enlarged, and x-ray examination showed advanced ossification. The bone

FIG. 6. *Case 1.* Note the advanced development of the epiphyses and the increased size.

age was 4 years, with irregularities in the chronologic appearances of the ossification nuclei. The vitamin products which had been taken regularly were discontinued at this time, and the parents were advised to consult an endocrinologist.

The family history was of no importance. The parents were cousins. The patient was the fourth of 5 children; the second child, who was underweight, died at the age of 11 months, with constipation and anorexia.

The patient had had furunculosis of the head and pertussis at the age of 1 year, with occasional bloody sputum.

Physical examination: The boy was brachycephalic with projecting zygomas, and had accentuated superciliary arches, fanlike ears (more pronounced on the right), a pointed jaw, and a thick neck with marked horizontal skinfolds. The skin was dry and flabby, with little fat and turgor. The back, limbs and pubis were covered with thin golden hairs. The abdomen was prominent, the collateral circulation was plainly outlined, and the navel projected. There were no signs of ascites. The spleen was not palpable, but the x-ray technic of Benhamou revealed that it was slightly enlarged. With the patient under general anesthesia it was possible to feel the liver, which was enlarged and of a firm consistency. This was principally true of the left lobe, which was six fingers longer under the xiphoid process.

The muscles of the arms, legs and abdomen showed mature development, with the muscular masses well differentiated. However, the normal muscular tonus did not correspond with the increase in the muscular mass. The veins of the limbs were thick and pro-

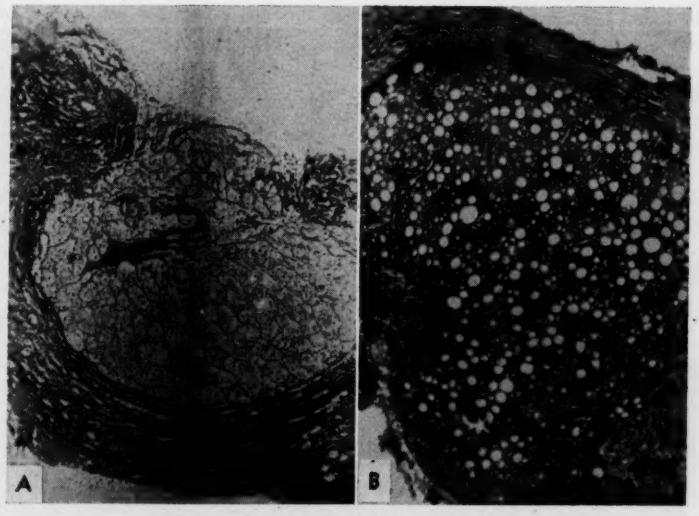

FIG. 7. **A.** *Case 1.* Intense fibrosis circumscribing an islet of liver cells. (Reticulin.) **B.** *Case 1.* Infiltration of the liver by neutral fats. (Milky serum. Total lipemia 1,200 mg. per 100 cc. Blood cholesterol concentration, 160 mg. per 100 cc.)

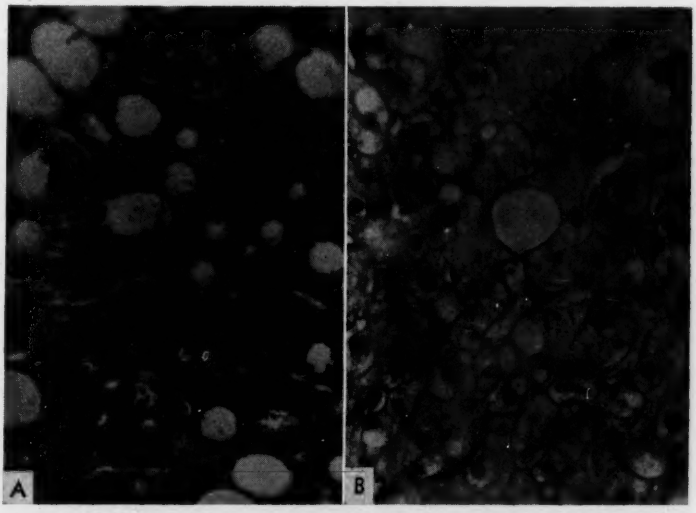

FIG. 8. **A.** *Case 1.* Distribution of the glycogen in the liver (McManus-Hotchkiss method). In black appear the regions in which the parenchyma is the richest in glycogen. **B.** *Case 1.* Saliva-McManus-Hotchkiss method. Complete removal of the positive Schiff material.

FIG. 9. *Case 1.* Bundles of muscles fibers separated by thick sections of adipose connective tissue.

FIG. 10. *Case 1.* Showing bundles of intersecting and coarse elastic fibrils. (Paraldehyde of Gomori.)

jecting. Knees, ankles, elbows, wrists and the epiphyses of the phalanges were bulbous, the latter giving a gross appearance to the fingers. Hands and feet were big and coarse. The genitals were highly developed for the age—a large thick penis, folded scrotum, and testes the size of those of a 7-year-old boy. A coccygeal dimple also was noted. The weight was 15.7 Kg. (the norm for this age in Brazil is 12.8 Kg.), and the height was 94.5 cm. (the norm for this age in Brazil is 84 cm.), The span was 95 cm. and the pubis-ground measurement, 43 cm.

A more recent radiologic examination made at the age of 2 years and 2 months revealed a bone age of 6 years, with irregularities in the chronologic appearances of the ossification nuclei. The sella turcica was normal.

Laboratory findings[2]*:* The cerebrospinal fluid was normal. Kahn, Kline and Wassermann tests all gave negative results.

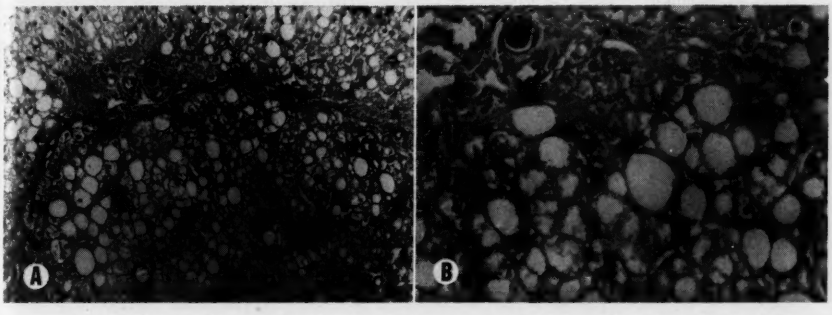

FIG. 11. **A.** *Case 2.* Cirrhosis and fatty infiltration of the liver. **B.** *Case 2.* Fatty infiltration of the liver in more detail. (Milky serum. Total lipemia 1,900 mg. per 100 cc. Blood cholesterol concentration, 144 mg. per 100 cc.)

The serum was milky. Blood chemistry (all values, per 100 cc. of plasma) showed: calcium 11.5 mg.; cholesterol (Bloor) 144 mg.; total lipids (G. Villela) 1.9 Gm.; protein (Gornal, Bardawill and David)—total 10.2 Gm., albumin 7.4 Gm., globulin 2.8 Gm.; urea 20 mg.; creatinin 1.4 mg.; and uric acid 3.5 mg. In a 24-hour specimen, urinary creatinine was 900 mg., 17-ketosteroids (Callow) 2.3 mg. and FSH (Klinefelter) 6.6 M.U.

Hemogram: red cells 4,850,000/cu. mm.; hemoglobin 15 Gm. (88 per cent); hematocrit 42 per cent; platelets 286,000/cu. mm.; color index 0.91; mean corpuscular hemoglobin 31 micro-micrograms; and mean corpuscular volume 87 cubic microns. White cells 8,800/cu. mm.; basophils 0; eosinophils 3.5 per cent; neutrophils 37 per cent (stabs 2 per cent, segmented 35 per cent); lymphocytes 42.5 per cent; and monocytes 17 per cent. Prothrombin time was 15.5 seconds and prothrombin activity 67.5 per cent (normal plasma control, 14 seconds).

In the glucose tolerance test, fasting blood sugar was 121 mg. per 100 cc. In 10, 30, 60, 90 and 120 minutes after the ingestion of 50 Gm. of glucose it was, respectively, 114, 114, 102, 105 and 108 mg. per 100 cc.

Histopathology of the liver: The histopathologic examination of the serial sections indi-

[2] See Addenda.

cated profound alterations in the structure of the liver (Fig. 11). There was an increase in fibrous tissue, not only between the lobules but, in certain regions, within the lobules.

Most of the liver cells on the paraffin slides were vacuolated; they had either a single and voluminous vacuole or smaller and numerous ones. The cells of the lobular periphery showed the greatest damage and frequently showed a single voluminous vacuole. A few lymphocytic cells were found here and there in the portal spaces, and in the interlobular fibrous tissue.

The search for lipids with Sudan III, Sudan IV and Sudan Black showed lipid deposits in the vacuoles.

Histopathology of the skin: Examination of skin fragments in serial section showed a normally formed epidermis with a discrete pigmentation of the basal layer, a slight relaxation of the collagen fibers of the derma, and the presence of numerous sweat glands. There were no xanthelasmic histiocytic cells.

Histopathology of muscle: Microscopic examination of muscle tissue showed the presence of several striated muscle fibers separated by delicate connective septa in which vessels and nerves were seen. The muscle cells were defined clearly and no infiltrations of adipose tissue were seen.

Diagnosis: Marked steatosis of the liver, with fibrosis.

COMMENT

It was impossible to arrive at a correct diagnosis. All syndromes having some features in common with these cases were excluded. Possibly there is some relationship to pituitary hyperfunction.

As the 17-ketosteroids were somewhat above the normal range, the possibility of an adrenal or testicular hyperfunction was considered. The testes were normal in relation to the *apparent* age in both cases; the penis was slightly larger. However, pubis and axillary hair were not abundant, there was no acne, and the voice was infantile.

Just as an hypothesis, we submit that the mild signs of hyperandrogenism may have been the consequence of an increase in LH, and the other manifestations may have been the result of the action of somatotropin. Considering the possibility that LH may also be produced by eosinophils, it may be that in the present cases we are dealing with a panhypereosinophilism.

Without making any positive statement, we found it interesting to compare the principal features of these cases with the multiple effects of somatotropin.

Gigantism: There is an obvious relationship.

Hyperlipemia and fatty infiltration of the liver: Extracts of the anterior hypophysis increase lipemia and produce a marked fatty infiltration of the liver, as Foglia and Mazzocco (1), Munoz (2) and De Robertis (3) demonstrated in 1938. Li, Simpson and Evans (4) definitely concluded (1949) that in rats this action is due to somatotropin.

Hyperproteinemia: Working with dogs, Goldberg (5) obtained an increase in the level of all plasma proteins, even with extremely small doses of anterior pituitary extract (1934, 1938). The same result in dogs was obtained by Campbell, Lei and Davidson (6) with the purified growth hormone (1951).

Disturbances of carbohydrate metabolism: The first case (with a hyperglycemic glucose tolerance curve) must be considered separately from the second case (with a hypoglycemic glucose tolerance curve).

In Case 1, the "Houssay-dog", "Young's" diabetes, and the repeated clinical coincidence of acromegaly and diabetes would explain the hyperglycemia.

In Case 2, the child was four years younger, and it is conceivable that the disease was in an earlier stage.

Richardson and Young (7) state: "The proliferation of the islet cells can precede, in the dog, exhaustive or degenerative alterations which accompany the appearance of a permanent diabetes."

Young (8) considers "the possibility that the diabetogenic, pancreatotropic and somatotropic actions of the anterior hypophysis extracts are due to the same substance."

Conn and Louis (9) showed that "the same extract of anterior pituitary gland which is eventually diabetogenic in dogs quickly intensifies the hypoglycemia of human patients suffering from organic hyperinsulinism," and concluded "that there exists in 'diabetogenic extracts' of anterior pituitary gland an insulotropic factor which exerts its effect directly upon the islands of Langerhans, and that this factor may be identical with that which initiates islet cell degeneration in some species."

X-ray therapy: In the second case we tried x-ray therapy of the pituitary —20 sessions each of 192 r, total 3,840 r (deep dosage: 1,300 r). The liver returned to normal size, the abdomen became less prominent, the turgescence of the muscles and veins of the limbs was reduced, and the hyperlipemia fell from 1,900 mg. to a normal level of 620 mg. per 100 cc. The proteinemia, however, remained at the same high level (10.5 Gm. per 100 cc.), and the glucose tolerance curve was still of the hypoglycemic pattern.

Coccygeal dimple: Both patients presented "foveola coccygea" (Fig. 5). Rosser (10), in 1928, wrote that in the production of coccygeal dimple "dysfunction of the pituitary plays an important role, inasmuch as many of these cases occur in large and peculiarly developed overweight boys."

SUMMARY

The author presents 2 cases of a syndrome characterized by an infantile acromegaloid gigantism, great muscular masses, hepatosplenomegaly,

cirrhosis and fatty infiltration of the liver, hyperlipemia with a milky blood serum, hyperproteinemia, and disturbances of the carbohydrate metabolism.

ADDENDA

Case 1. This patient died in August, 1953, with massive intestinal hemorrhage, which lasted for three days. The assisting physician (it happened in the hinterland) informed us that the liver had diminished in size, and that the patient had been going regularly to school. A week before his death his height was 142 cm. (normal in Brazil for his age, 116 cm.). It was not possible to perform an autopsy.

Case 2. The most interesting feature is that after two hypoglycemic glucose tolerance curves (in March 1953 and in June 1953), the repeated tolerance curve in September 1953 was hyperglycemic in type, as follows: fasting blood sugar level, 117 mg. per 100 cc.; 10, 30, 60, 90 and 120 minutes after ingestion of 50 Gm. of glucose, respectively 129, 133, 142, 148 and 153 mg. per 100 cc.

If the pathogenesis of this syndrome should, by any chance, be concerned with an increase of somatotropin, it is possible that this patient passed from the insulotropic stage of somatotropic activity to the diabetogenic stage, as suggested by Young and by Conn, and as we had predicted in our case.

On April 18, 1953, serum analyses showed: calcium 9 mg., inorganic phosphorus 5.8 mg., and alkaline phosphatase 7 Bodansky units per 100 cc.

The patient is doing well (October 1953).

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LACK OF EFFECT OF HYPOPHYSECTOMY UPON METASTATIC ADRENOCORTICAL CARCINOMA WITH CUSHING'S SYNDROME: A CASE REPORT*

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THE consideration of hypophysectomy as a palliative measure in the therapy of malignancy was stimulated by the appearance in 1950 and 1951 of a series of papers from Evans' laboratory showing that the injection of pituitary growth hormone over prolonged periods approximately doubled the incidence of spontaneously appearing neoplasms in rats (1), whereas in hypophysectomized animals observed for a comparable period of time, with or without growth hormone injections (2), far fewer tumors developed than in control animals.

An interesting review of the literature by Archer (3) included 13 instances of hypopituitarism and coexisting neoplasms. Of these, 4 were cancers of the stomach, 3 of the bronchi, 2 of the uterine cervix, and 1 each of esophagus, colon, mediastinum and thyroid (sarcoma). Hence, in humans, the existence of hypopituitarism does not preclude the appearance of cancer.

The effect of hypophysectomy, once a neoplasm is established, has not, to our knowledge, been investigated in animals. In humans, a report by Shimkin *et al.* (4) of hypophysectomy in a young man with metastatic malignant melanoma appeared last year. In the nine weeks which the patient survived postoperatively, clinical evidence of hypopituitarism supervened. At death the findings, apart from the evidences of neoplasm, included complete absence of hypophyseal tissue with the exception of one focal area containing about two dozen cells, and atrophy of the target endocrine organs. No significant effect of the operation upon the growth of the neoplasm could be demonstrated.

More recently Luft and Olivecrona (5, 6) have described the application

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of this operation in 12 patients with malignancy. Of these, one with a chorionepithelioma and another with a breast neoplasm were strikingly improved. An additional patient has been reported by Perrault *et al.* (7).

The subject of the present case report was a young man with an evident metastatic adrenocortical carcinoma, presenting the clinical picture of Cushing's syndrome. Hypophysectomy was undertaken in this patient in the hope that 1) the growth of the neoplasm might, like that of normal adrenal tissue, in some measure be dependent upon the endogenous production of ACTH, and/or 2) the operation might serve nonspecifically to inhibit tumor growth. The results of the operation were clinically disappointing. Indeed, the paucity of demonstrable change in endocrine function was striking during the seven weeks of life following the operation. Since no remaining pituitary tissue could be demonstrated at autopsy these unusual findings were considered to be related to the persistence of hormone production by the autonomous malignant tissue, which in one sense maintained the patient's life, while in another it hastened his demise.

C.R. (P.H. unit #072193), was a 26-year-old married man who was referred to Dr. Henry Aranow, Jr., and by him to the wards of the Columbia-Presbyterian Medical Center in November 1951, with a history of progressive weakness and a 29-pound weight gain over a period of four months. Hypertension had been noted by his family physician two and a half months after onset of symptoms. For this, the patient was given a 1,000-calorie salt-poor diet, during which treatment he lost 20 pounds in ten days. At the time of his hospitalization further questioning brought out the additional points that his weight gain had been limited to the head and trunk, and that there had been changes in his skin; his face had become ruddy and dry, and he had demonstrated a tendency to easy bruisability, with acneiform lesions. He admitted nocturia, averaging three times nightly, but he specifically denied having headaches, loss of libido or backache.

On physical examination he appeared a pleasant, intelligent young man of asthenic build, his height being 184 cm. and weight 67.7 Kg. In contrast to his over-all leanness, his face appeared rounded and chipmunk-like, with a ruddy complexion. Body hair was present in normal male distribution; however, dryness and thinness of the skin were evident. Careful search revealed three small purple striae, one inside the left thigh, and one above and posterior to each iliac crest. His blood pressure was 190/120, pulse 80, and temperature 99° F. per rectum. The rest of the findings on physical examination were within normal limits. Specifically his eyegrounds appeared normal. There was no evidence of cardiac enlargement or decompensation, *i.e.*, no basal rales, peripheral congestion, hepatic enlargement or ankle edema.

Initially laboratory studies revealed the following: hemoglobin 15.0 Gm., erythrocyte count 5.6 million per cu. mm.; white blood cell count 11,000 per cu. mm.; polymorphonuclear leucocytes 82 per cent; lymphocytes 16 per cent, monocytes 2 per cent. The quantitative eosinophil count was 25 per cu. mm., and the erythrocyte sedimentation rate was 7 mm. in one hour. Urinalysis: specific gravity 1.023; no albumin or glucose; microscopic, unremarkable. The Mazzini test for syphilis gave negative results. The concentration of blood sugar, fasting, was 103 mg. per 100 cc. After an oral dose of 100 Gm. of glucose the blood sugar level rose at one-half hour to 204, at one hour to 303, and at

two hours to 339, with a final value at three hours of 213 mg. per 100 cc. Serum sodium concentration was recorded as 139.1 and 144.1 mEq., potassium as 2.6 and 2.8 mEq., carbon dioxide 35.8 and 37.3 mEq. and chlorides 96.1 and 98.7 mEq. per liter; urea nitrogen 11 mg., calcium 9.9 mg., phosphorus, 2.7 mg., and alkaline phosphatase 3.5 Bodansky units per 100 cc. The initial value for urinary 17-ketosteroid excretion was 35.5 mg. (normal, 8–20) in twenty-four hours, and for "corticoids" as determined by the formaldehydogenic method, 2.8 (normal, 0.5–1.5) mg. in twenty-four hours.

An electrocardiogram and an electroencephalogram showed nothing abnormal. A benzodioxane test gave no evidence that the hypertension was due to excess amounts of circulating epinephrine or norepinephrine.

X-ray examination of the skull showed no abnormality of the sella turcica and no demineralization, nor was there any evident demineralization of the thoracic spine. The initial x-ray films of the chest showed no cardiac enlargement and normal lung fields. By intravenous pyelography, there was a suggestion of an irregularity of the left renal pelvis. The introduction of air retroperitoneally outlined a mass above the left kidney, which was even more clearly delineated by an aortogram.

Course: After preoperative preparation with 100 mg. of cortisone acetate (given intramuscularly at twelve-hourly intervals beginning thirty-six hours before the procedure) and 5 mg. of desoxycorticosterone acetate (given the morning of operation), Dr. George Cahill removed the left adrenal and tumor on December 11, 1951. The tumor weighed 407 Gm. and Dr. M. M. Melicow reported that histologically it appeared malignant. Nests of cells were noted extending out beyond the tumor capsule. A surprising finding was the histologic pattern of the adjacent "normal" adrenal tissue; instead of the anticipated atrophy, this tissue appeared hyperplastic with a preponderance of "foam cells."

The postoperative period was essentially uneventful and at the time of discharge on December 27, 1951, fifteen days after operation, the patient was feeling well and was receiving no medication. Although the persistence of significant hypertension raised the question of possible metastases, comfort was derived from the normal serum electrolyte values at the time of discharge (sodium 141.2, potassium 3.5, carbon dioxide 30.0 and chloride 100.0 mEq. per liter), along with a fall in the urinary 17-ketosteroid excretion to 14.5 mg. per twenty-four hours.

At home during the ensuing weeks his symptoms recurred and, in addition, he had pain in the mid-thoracic spine. When seen in clinic at the end of a month's time, his blood pressure was recorded as 170/120. Plethora persisted and new developments were tenderness over the seventh thoracic vertebra and hepatomegaly to the extent of two fingerbreadths. These were considered indicative of metastases and the patient was readmitted to the hospital.

Studies during this second admission revealed the development of marked abnormalities in serum electrolyte concentration (sodium 153.6, potassium 1.9, carbon dioxide 41.8 and chlorides 94.7 mEq. per liter), the appearance of frank diabetes (fasting blood sugar levels, as high as 222 mg. per 100 cc., and a daily insulin requirement of 20 to 35 units to control partially the heavy glycosuria), and a return to a markedly increased 24-hour urinary excretion of 17-ketosteroids (51.6, 40.8, 39.3 and 53.1 mg.). Apart from this physiologic evidence of excessive and abnormal adrenal function, there were confirmatory findings of metastatic disease, which included x-ray shadows suggestive of pulmonary metastases and of an osseous lesion in T_7, and a bromsulphalein retention of 20 per cent thirty minutes after the injection of the dye. The neutral extract of a 24-hour urine specimen was chromatographed according to the Devis (8) modification of the Dingemanse method. The eluates showing the highest concentration of Zimmermann

chromogen were analyzed by infrared spectrophotometry through the courtesy of Dr. Seymour Lieberman (Fig. 1). Dehydroisoandrosterone was found in no higher concentration than in the urine of the normal male, the values for which are included in the figure for comparison. This steroid is often found in high concentrations in certain adrenal tumors. Androsterone and etiocholanolone were present in higher concentration in this patient than in a normal man. More significant, however, was the finding of appreciable

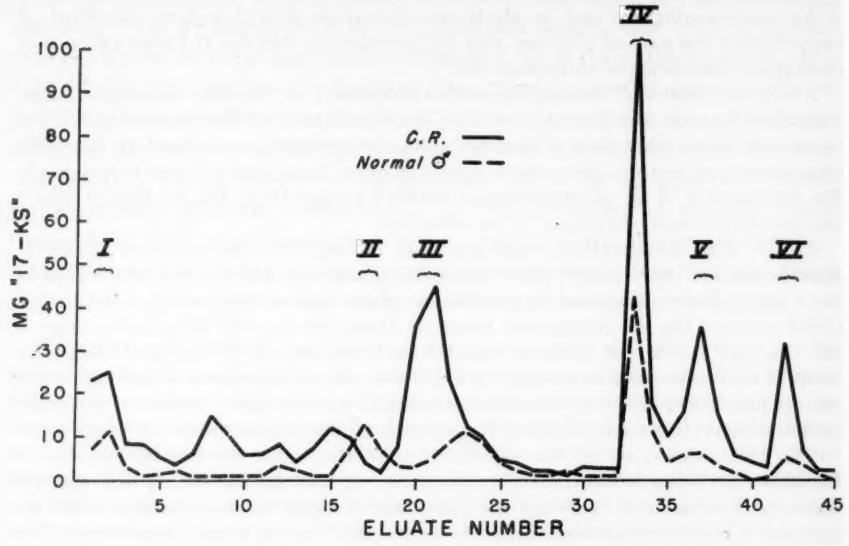

FIG. 1. Chromatogram of the neutral urinary extract of Patient C.R. compared with that of a normal 24-year-old male. In C.R. analysis of peak I showed nonspecific chromogen but no steroid; peak II, dehydroisoandrosterone; peak III, androsterone; peak IV, etiocholanolone; and peak V, 11-keto-etiocholanolone. Usually peak VI is a nonspecific chromogen, but in Patient C.R. it was identified as 11-keto-etiocholanolone.

quantities of 11-keto-etiocholanolone, as contrasted with the excretion in the normal male. This steroid is a known degradation product of cortisone or 17-hydroxycorticosterone.

The patient was given 100 milligrams of cortisone over a four-day period, to determine whether the resulting suppression of pituitary function with reduction in endogenous ACTH excretion would influence the adrenal steroid output of the metastases, *i.e.*, whether the tumor remained to any extent under pituitary influence. The third and fourth day of cortisone injections his 24-hour urinary excretion of 17-ketosteroids was 56.1 and 63.0 mg., respectively, indicating no downward trend in steroid output. In this he differed from patients in whom Cushing's syndrome is associated with adrenal hyperplasia (9).

Radiation of the pituitary was contemplated, although the foregoing results augured poorly for the likelihood of its being beneficial, However, before such therapy could be instituted, an acute organic psychosis developed, with associated schizophreniform features which necessitated his being admitted to the New York Psychiatric Institute.

While there, on an unrestricted carbohydrate intake, a dose of standard insulin up to 80 units per day was required partially to control his glycosuria.

At this time hypophysectomy was suggested as an admittedly desperate therapeutic approach. Accordingly, on February 28, 1952, Dr. J. L. Pool performed a complete hypophysectomy through a right frontal craniotomy.

Sections of the excised anterior pituitary showed "Crooke's" changes in the basophils. The operation lasted four hours, and from a neurologic point of view the patient's course was eminently satisfactory both during and throughout the postoperative period. Dilantin and phenobarbital were given postoperatively and penicillin prophylactically. To protect against possible hypoglycemia after hypophysectomy the patient was given cortisone preoperatively and this was continued for three days postoperatively, in diminishing dosage. Subsequently, in view of persistent heavy glycosuria, it was omitted; for the remaining six and one-half weeks of his life no adrenal substitution therapy was given.

Immediately after operation insulin was administered with some trepidation but it soon became apparent that sensitivity to this hormone had not been markedly altered, and 10 to 20 units of insulin were required daily until the day of his death. Eighteen blood sugar determinations were made during the seven-week period following hypophysectomy, during which the fasting values varied from 177 to 444 mg. per 100 cc. Throughout the period, intermittent glycosuria persisted.

Despite the fact that no adrenal substitution therapy was employed after the third postoperative day, serum sodium values did not fall below normal, and twenty-four hours prior to death a value of 135.3 mEq. per liter was obtained (Table 1). The serum potassium concentration rose from the strikingly low preoperative levels and terminally was 4.7 mEq. per liter.

Throughout the post-hypophysectomy period the excretion of 17-ketosteroids continued to be strikingly elevated. A single determination of urinary "corticoids" made four weeks after hypophysectomy was 8.4 mg. in twenty-four hours, a markedly elevated value.

Postoperatively the urine volume rose to 4,300 cc. in the first twenty-four hours; and when heavy glycosuria was not present, the specific gravity was extremely low. Posterior pituitary extract was given in doses of 5 units, and the urine volume diminished somewhat although, because of incontinence, accurate measurements could not be obtained. It was found expedient, both from the point of view of the patient's comfort and as a practical help in nursing care, to continue posterior pituitary extract, which was given twice daily. During this regimen, urinary output ranged as high as 3,700 cc. in the absence of heavy glycosuria.

Attempts to evaluate thyroid function were of necessity limited. The patient's emotional lability made it difficult to measure his basal metabolic rate. However, in the fourth week after hypophysectomy a fairly satisfactory pair of tracings averaged −1 per cent. Serum cholesterol concentration (on two occasions in the third and fourth postoperative week) was low, perhaps related to his widespread malignant disease. His mental abnormalities precluded the cooperation necessary to obtain an estimation of radioactive iodine uptake.

The patient continued to deteriorate slowly following the hypophysectomy, although his appetite remained good. His mental abnormalities fluctuated from day to day but the basic aberration remained unchanged. Ascites developed, and the hypertension decreased gradually postoperatively and in the week prior to death ranged from 140/86 to 130/84. In the seventh postoperative week frank pulmonary edema occurred; in spite of

TABLE 1. CLINICAL AND LABORATORY DATA ON PATIENT C.R., PRIOR TO, AND AFTER TOTAL HYPOPHYSECTOMY

	Three wks. preop.	Immediately preop. Feb. 27	Postoperative week 1	2	3	4	5	6	7
			Mar. 5	Mar. 12	Mar. 21	Mar. 25	Apr. 1	Apr. 7	Apr. 15 (day before death)
Weight, Kg.	60.5		59.85	57.78	59.0	60.0	58.19		
Blood pressure	180/120		180/120	152/94	148/86	140/88	140/100	150/90	
Cortisone acetate, daily dose (mg.)			75–25	0	0	0	0	0	0
Insulin, daily dose (units)	0		45*	24*	20*	0	15†	20†	10†
Glycosuria (max.)	+++		++++	++++	++++	++++	+++	++++	0
Fasting blood sugar (mg./100 cc.)	222	308‡	384	213	179	213	204	225	211‡
Urine specific gravity, (max.)§			1.010	1.004	1.015		1.012		1.015
Post. pituitary hormone, daily dose (units)			5	5	10	10	10	10	0
Serum sodium (mEq./L.)	153.4	137.0	137.2	143.0	144.1	140.9	144.2	138.3	135.3
potassium (mEq./L.)	2.3	2.9	3.8	2.7	2.8	3.5	3.5	2.9	4.7
CO_2 (mEq./L.)	39.3	29.5	38.2		33.8		33.9		
chlorides (mEq./L.)	98.0	96.2	97.4		94.8		100.3		
Urinary 17-ketosteroids (mg./24 hrs.)	63.0		41.6	45.1			44.0		
Urinary corticosteroids (mg./24 hrs.)						8.4			
Basal metabolic rate (%)						−1			
Serum cholesterol (mg./100 cc.)				117	138				
Blood vol. (cc.)			4,450			4,760			
Plasma vol. (cc.)			3,000			2,950			
Hematocrit (%)			32.6		38.5	38.1			

* Standard insulin.
† NPH insulin.
‡ Not fasting.
§ Maximal gravity recorded when urine contained no more than "+" glucose and when eight hours or more had elpased since an injection of posterior pituitary hormone.

treatment with the usual measures, this edema persisted and led to his death twenty-four hours later.

Autopsy findings revealed 2,500 cc. of bloody ascitic fluid and great clusters of grape-like tumor nodules which hung from the peritoneal surfaces. The heart weighed 350 Gm. and the aorta was surprisingly free of atheromata. The lungs were edematous. A few subpleural nodules were present in both lower lobes. The liver weighed 4,440 Gm., and on cross-section there were myriads of tumor nodules of all sizes up to 8 centimeters in diameter. The pancreatic tissues appeared normal. The right kidney weighed 175 Gm.; the left, though not weighed, appeared to be about the same size.

The whole region of the left adrenal was filled with large masses of white and reddish tumor, packed together like fieldstones in a wall. No adrenal tissue was unequivocally identified on the left. The right adrenal was entirely free of tumor, and was reduced in size (weight, 3.5 Gm.). On section the cortex was obviously thinner than usual, and bright orange in color; the medulla appeared normal.

The prostate was small, measuring perhaps 2 cm. in diameter. The testes were small and soft. The thyroid weighed 15 Gm. and was slightly nodular. Three parathyroids were found; they were of normal size and appearance. No osteoporosis was grossly detectable.

The brain, apart from the evidence of the operative procedures, was grossly normal. The pituitary fossa appeared empty.

Microscopic examination: The skin showed atrophy of the dermis and epidermis. The heart and aorta were essentially normal. In the lungs the presence of pulmonary congestion was confirmed; some areas showed pneumonia and foci of carcinoma. The liver, in addition to multiple areas of tumor invasion, showed a number of foci of necrosis, mostly central. Most of the tumor cells on section resembled cortical cells depleted of lipid, although there were undifferentiated variants.

The right adrenal gave the impression of a miniature but perfect gland, except for the marked vacuolization of nearly all cells of the cortex. The medulla was not remarkable. The prostate showed some piling up of epithelium in the glandular lumina. In the testes, basement membranes were thickened and, although there was some cell division among spermatocytes, no spermatozoa were seen. The thyroid contained follicles of medium size, composed of low cuboidal cells with abundant colloid. In the pancreas, islets of Langerhans seemed unusually abundant. The parathyroids were not remarkable. Brain: In sections of the floor of the sella turcica no pituitary tissue was found. The leptomeninges about the tuber cinereum and infundibulum contained some red blood cells, lymphocytes and small numbers of large mononuclear cells. There were numerous small recent hemorrhages in the tuber cinereum, and the adjacent floor of the third ventricle showed a fresh proliferation of ependymal granulations. The supraoptic nucleus on one side showed a decrease in the total number of its nerve cells and an associated astrocytosis. The same nucleus on the other side was disrupted by a series of small recent hemorrhages, and was rather edematous, as was the adjacent superficial aspect of the dorsal portion of the optic tract. There was shrinkage of many of the nerve cells in the corpora mammillaria. The paraventricular nuclei bilaterally showed no unusual change.

DISCUSSION

Hypophysectomy was advocated in this patient, with the hope that the neoplastic tissue would prove to have that dependency upon an endogenous supply of ACTH which characterizes the normal adrenal cortex. Were this

the case, regression of the tumor could be anticipated following surgical removal of the pituitary. This hope was not substantiated postoperatively, and the patient's downhill course appeared unaffected by the procedure. The alternate possibility that tumor growth might be depressed nonspecifically by the induction of a hypopituitary state remained unexplored, since the continued production of steroids by the metastatic cells prevented the establishment of panhypopituitarism.

An astonishing feature of this patient's course was the sustained ability of the highly malignant tissue to elaborate a variegated steroid pattern. Since autopsy indicated that the pituitary had been satisfactorily extirpated and the one remaining adrenal was atrophic, as was to be expected from the deprivation of all ACTH, it is evident that the source of the adrenal steroids was the metastatic tumor tissue.

Perhaps the most striking evidence of steroid activity was the fact that hypophysectomy had no significant effect upon the diabetes. Heavy glycosuria persisted, insulin requirements were essentially unchanged, each of the eighteen blood sugar determinations made postoperatively showed significantly elevated values, and no definitive insulin shocks were encountered. This condition obtained, although no adrenal substitution therapy was given for the last six weeks of life. It should be mentioned that the first hypophysectomy involving a normal pituitary, reported by Chabanier *et al.* in 1936 (10), was undertaken in a young boy with severe diabetes. Postoperatively his daily insulin requirement fell from about 140 units to only 80 units. However, unlike the patient in the present report, no autopsy was performed; hence, the completeness of the hypophysectomy was not established. Certainly since the classic studies of Houssay, the ameliorating effect of hypophysectomy on pre-existing diabetes has been well recognized, and it is well known that recurrent hypoglycemia may present a difficult problem in the management of hypopituitary patients. In view of this, the physiologic activity of this patient's neoplastically produced "steroids" is the more impressive.

Another evidence of the physiologic activity of steroids elaborated by the metastatic tumor was the maintenance of serum electrolyte levels in the absence of added salt, or steroid replacement therapy. The abnormalities associated with excessive cortisone were intermittently present postoperatively—the carbon dioxide content of the blood was consistently elevated and the potassium and chloride levels were frequently reduced below normal. The sodium concentration fluctuated, but was for the most part normal.

A feature of hypophysectomy which has recently been investigated experimentally in Evans' laboratory by Garcia *et al.* (11) and by Van Dyke *et al.* (12) is the reduced blood and red cell volume. These workers have

shown that whereas this abnormality is not corrected in rats by the administration of growth hormone, it is corrected by the administration of ACTH with the resulting normal amounts of circulating cortical hormones. In the present patient it was of interest to note that no change in blood volume (Table 1) could be demonstrated between the first and fifth weeks after hypophysectomy.

Corroborating these physiologic evidences of hormone secretion were the studies on urinary excretion of steroid metabolites. On the basis of the chromatographic studies it is possible to say that although quantitatively in great excess, the pattern of steroid secretion in this patient conformed to that seen in normal subjects, except that there was a higher percentage of 11-keto-etiocholanolone (Fig. 1).

Following the operation there was a significant reduction in blood pressure, not to normal values but certainly to a more normal range than had existed previously. How much this should be ascribed to the hypophysectomy is problematical. Certainly all other evidences of hyperadrenalism persisted and yet it seems unlikely that the reduction could have been the result of the inanition associated with malignancy, since the patient held his weight and continued to eat well. One last but particularly tragic aspect of the persistent hyperadrenalism was the continued psychotic condition.

Little comment can be made upon the function of the posterior pituitary, because of the paucity of data. Immediately after hypophysectomy the patient's fluid turnover was markedly abnormal; undoubtedly part of this was due to heavy glycosuria, but when glycosuria was intermittently absent the patient continued to excrete large volumes of urine of low specific gravity. He was treated for this condition with posterior pituitary extract, until his death. In the latter weeks, a rise in urinary specific gravity to as high as 1.015 suggested some amelioration of the diabetes insipidus.

Even less can be said regarding thyroid function. The normal basal metabolic rate obtained in the fifth week after hypophysectomy is out of line with the gross anatomic evidence of atrophy of this gland at autopsy. However, the patient was paranoid and in a borderline state of cardiac failure; these factors may have prevented his achieving a basal state.

SUMMARY

This report describes a 26-year-old man with metastatic adrenocortical carcinoma, accompanied by typical Cushing's syndrome. No significant change in the clinical picture occurred after complete removal of the pituitary, with the exception of the appearance of a state resembling mild

diabetes insipidus. No steroid replacement therapy was given subsequent to the first week after hypophysectomy. Nevertheless, the severe diabetes mellitus, normal serum electrolyte values or values suggesting hypochloremic alkalosis, markedly elevated urinary excretion of 17-ketosteroids and corticoids, and the pre-existing psychosis all persisted essentially unchanged.

Death occurred seven weeks after extirpation of the pituitary. An autopsy confirmed the completeness of the hypophysectomy and demonstrated moderate atrophy of the target endocrine glands—thyroid, testes and remaining adrenal. Metastatic malignant tissue was widespread.

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INFLUENCE OF ESTROGEN ON THYROID FUNCTION*

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THAT important relationships exist between thyroidal and gonadal function has long been suspected but, especially in man, these relationships have been only incompletely defined. Although attempts have been made to show that clinical or "subclinical" alterations in thyroid function cause menstrual or obstetric abnormalities, many studies have suffered from a lack of precise methods of evaluating thyroid activity. Also, many studies have been further hampered by a lack of data on the possible influence of ovarian hormones themselves on thyroid function or on the responsiveness of tissue to the circulating thyroid hormone.

The studies to be reported were undertaken to investigate the influence of estrogen on the activity on the thyroid. Preliminary investigations in this department (1) indicated that the administration of estrogen led to a rise in the serum precipitable iodine (SPI). These studies are now extended and an effort is made to elucidate the mechanism of the estrogen-induced rise in the SPI.

MATERIAL AND METHODS

Twelve men, whose ages ranged from 45 to 74 years, were given from 75 to 100 mg. of diethylstilbestrol daily. Five women, whose ages ranged from 21 to 70 years, were given 25 to 30 mg. of diethylstilbestrol daily and 2 women, aged 18 and 30 years, were given 5 mg. of Premarin (conjugated estrogens-equine) daily. None was acutely ill, although the majority were chronically ill with carcinoma of the prostate or breast, or suffered from osteoporosis.

A control serum precipitable iodine (SPI) determination was obtained in all instances and the course of the SPI followed at different intervals after beginning therapy. Also during this interval the patients were followed clinically for evidences of hyperthyroidism. In 10 it was possible to obtain accurate records of the resting pulse rate and in 5 it was possible to follow the basal metabolic rate. Estrogen therapy was discontinued in 10 patients and the course of the SPI followed thereafter.

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In 7 instances in patients with myxedema secondary to previous thyroidectomy or with anterior pituitary insufficiency, the effect of 30 mg. of diethylstilbestrol per day on the SPI was studied. All were receiving constant amounts of exogenous thyroid orally (from 0.06 to 0.18 Gm. of USP desiccated thyroid).

The SPI was determined by the method of Barker (2) as modified by Danowski and associates (3), utilizing the distillation technic. In our experience 95 per cent of normal individuals have an SPI level between 3.5 and 7.3 micrograms per 100 ml. of blood (mean, 5.1 micrograms). Also, our data and those of others (4) indicate that the SPI remains relatively constant in the same individual from time to time; variations greater than 1.0 microgram are unusual. Thus, for alterations of the SPI to be significant, as increments or decrements with time, these changes must be greater than 1.0 microgram and be consistent in direction.

RESULTS

In the 19 patients (12 men and 7 women) the SPI level rose in all instances during the administration of estrogen (Fig. 1). With few exceptions, the increments were generally linear, at least up to three weeks in time. During this interval the mean SPI had increased from 4.6 to 6.6

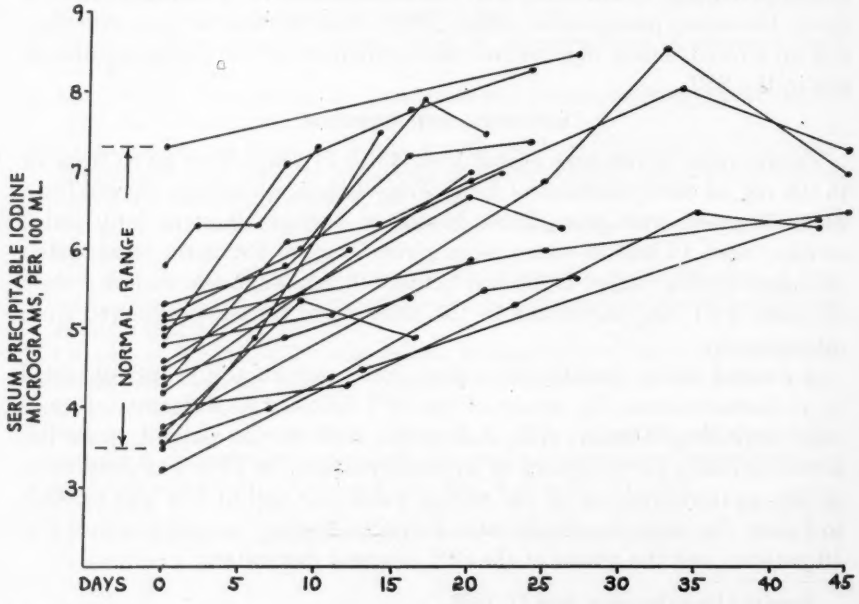

FIG. 1. Response of the serum precipitable iodine (SPI) to administered estrogen in patients with normal thyroid function. Estrogen was begun on day No. 1.

micrograms, and, in general, those individuals who had the higher control values tended to develop the highest SPI with administered estrogen. In those patients who received estrogen for longer periods, a "leveling off" in the increments was usually observed by the third or fourth week.

The minimal amount of estrogen required to elicit a significant response, or the amount needed to produce a maximal rise could not be clearly deduced from the data. In women, however, estrogen equivalent to 25 mg. of diethylstilbestrol produced as much of a rise as 100 mg. in men (maximal rise of 2.24 micrograms in the women, compared to 2.27 micrograms in the men).

The SPI gradually fell toward the normal control range (Fig. 2) in all instances when the estrogen was discontinued.

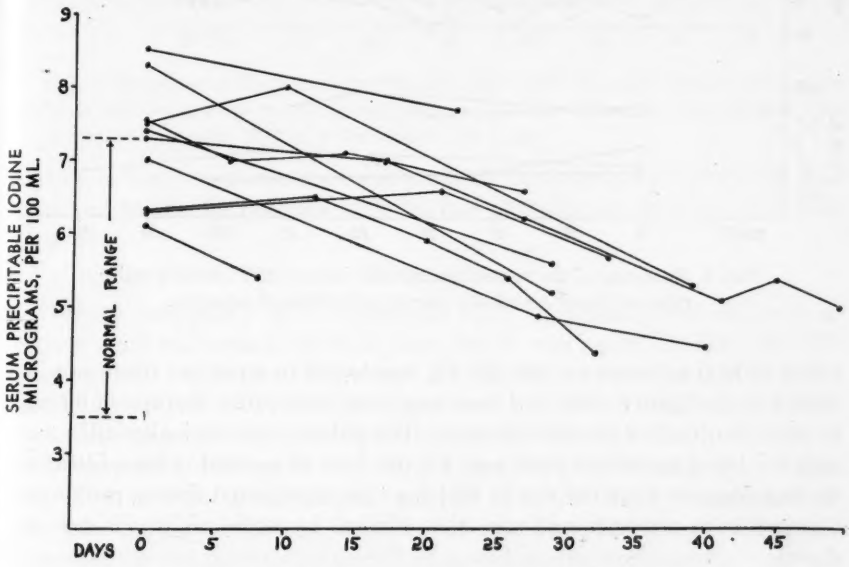

FIG. 2. Response of the serum precipitable iodine (SPI) when therapy with estrogen was discontinued.

In Figure 3 data are shown which indicate that in spite of progressive increments in the SPI, neither the resting pulse rate nor the basal metabolic rate was significantly altered. Although resting pulse rates were obtained daily on the 10 patients, for convenience in charting only the rates obtained every fifth day are recorded. Acceleration in pulse rate was not observed in any of the patients. In addition, none exhibited other signs or symptoms usually indicative of hyperthyroidism, in spite of the fact that in several instances the SPI was clearly in the hyperthyroid range. An SPI

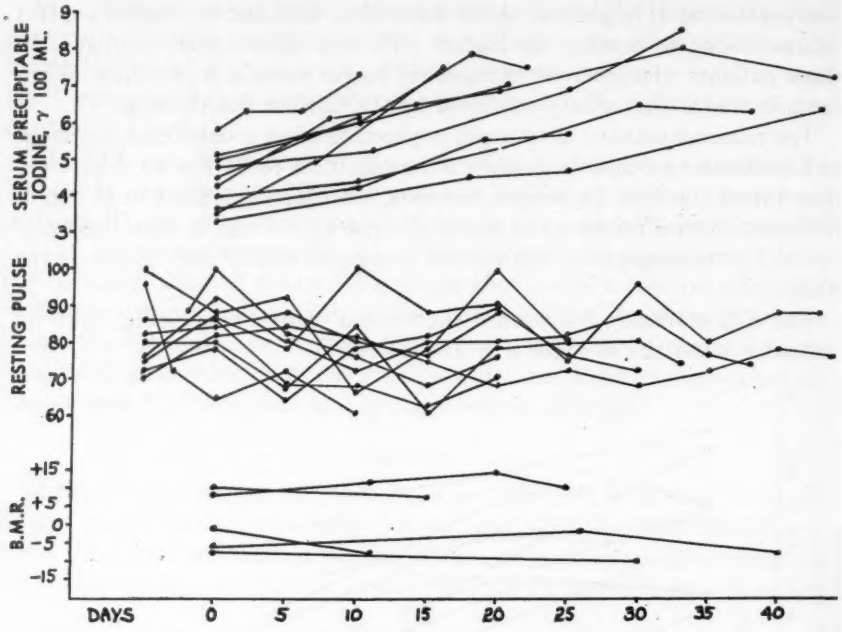

FIG. 3. Response of the serum precipitable iodine (SPI), resting pulse rate and basal metabolic rate to administered estrogen.

value of 10.0 micrograms per 100 ml. was found in a patient (data not included in the figures) who had been receiving continuous therapy of 60 mg. of diethylstilbestrol for three months. The patient was clinically euthyroid and her basal metabolic rate was +2 per cent of normal. This additional finding suggests that the rise in SPI may be maintained during prolonged therapy with estrogen and also that clinical hyperthyroidism is not induced.

An effort was then made to determine whether the increments of SPI were due to an actual increase in thyroid activity or due to an alteration in the mechanism of disposal of circulating hormone. Patients with anterior pituitary deficiency or myxedema secondary to previous thyroidectomy, who were maintained on constant amounts of exogenous thyroid, were given diethylstilbestrol in the same amounts that were used in the previous study. Presumably the thyroid glands of these individuals would not be able appreciably to increase their activity. It is seen in Figure 4 that in 5 of 7 instances no change in the SPI occurred. In one instance an unsustained rise occurred; in the other the rise was only 0.9 microgram. In neither case could the change be considered definitely significant. The

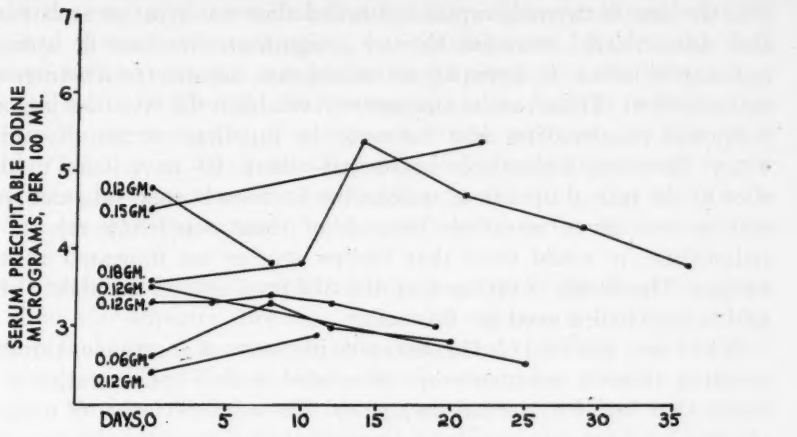

FIG. 4. Response of the serum precipitable iodine (SPI) to administered estrogen in patients with myxedema or anterior pituitary insufficiency. The daily maintenance dose (in grams) of desiccated thyroid is indicated in the figure.

results in the 7 cases contrast sharply with the invariable increments that occurred in the 19 patients with normal thyroid and pituitary function (Fig. 1).

DISCUSSION

Normal pregnancy is a physiologic state in which large amounts of estrogen are elaborated. Several years ago it was noted (5) that the SPI increases early in normal pregnancy, is maintained until after delivery and is not associated with the usual signs and symptoms which accompany increased concentrations of circulating thyroid hormone. The increment of SPI appeared to be thyroid hormone, in that it was extractable by butanol (6). The present investigations suggest that increased elaboration of estrogen during pregnancy may be at least one important factor responsible for the increments in SPI observed during pregnancy.

Our studies also support the concept that the activity of the thyroid is actually increased by estrogens, at least during the period when the SPI is rising. There was no clear evidence of an altered rate of disposal of the iodine bound to protein. Also, an intact anterior pituitary appeared to be necessary for the effect of estrogen on thyroidal activity to be noted. Although extensive data are not available, some investigations by others also support the idea that the activity of the thyroid is increased during pregnancy and by estrogens. In a few cases Pochin (7) showed that the rate of uptake of radioiodine by the thyroid is greater during pregnancy than in the nonpregnant state. Greer (8) stated that thyroid hyperplasia occurs in rats with persistent estrus and Reinecke and Saliman (9) showed

that the rate of thyroidal uptake of radioiodine was greatest at estrus and that administered estradiol elicited a significant increase in uptake of radioactive iodine. In hypophysectomized rats, administered estrogen had no such effect. These results and our own establish the fact that important reciprocal relationships exist between the functions of the thyroid and ovary. However, it should be noted that others (10) have found no difference in the rate of uptake of radioiodine between normal rats and spayed rats or rats given estradiol. Because of these conflicting reports with radioiodine, it would seem that further studies are indicated with this technic. The effects of estrogen on the SPI level and on the ability of thyroid to trap iodine need not be similar.

It has been reported (5, 11) that some instances of abortion or threatened abortion in early pregnancy are associated with a concentration of SPI below that found in normal pregnancy. The administration of exogenous thyroid has been advocated, to diminish the tendency to abortion. However, it has not been established whether the low SPI actually determines a miscarriage or is merely a symbol of another defect. This problem must be attacked in other ways before it is finally settled but the present studies suggest that inadequate production of estrogen could, at least in some cases, result in an inadequate rise in SPI. Both an adequate supply of estrogen and an adequate response by the thyroid to estrogen, or possibly to other factors, may be necessary for normal pregnancy. It is interesting that the Smiths (12) and others have advocated the use of estrogens rather than thyroid in cases of habitual abortion.

Both in the pregnant state and during the administration of large amounts of estrogen, values of SPI may be encountered which ordinarily would be associated with hyperthyroidism. Since available data indicate that the increment in SPI is composed of thyroid hormone (11) it must be concluded that under these circumstances there exists an increased tolerance to increased concentrations of circulating hormone. From our data we were unable to detect, during the rise of the SPI, clinical evidences of hyperthyroidism or significant alterations in the resting pulse rate or basal metabolic rate. Other data support the concept that in pregnancy also there is an increase in tolerance to thyroid hormone. Engstrom and his associates (13) and Astwood (14) concluded that the pregnant state appeared partially to ameliorate clinical hyperthyroidism. Also, Man and her associates (11) demonstrated that in pregnancy the SPI could be rapidly raised by the administration of thyroxine or thyroid to levels characteristic of hyperthyroidism, without the appearance of symptoms. The mechanism whereby this increase in tolerance to thyroid hormone is brought about is not established. A direct action of estrogen to decrease the effectiveness of circulating thyroid hormone on peripheral tissue is likely. Sherwood (15)

found a decrease of the metabolic rate of thyroidectomized male rats fed desiccated thyroid, when estrogens were administered. He felt the data indicated a direct hormone antagonism in the tissue between thyroid hormone and estrogen. The fact that estrogens depress the rate of respiration of tissue homogenates (16) is surely consonant with this concept. It is possible that this action of estrogen on cellular metabolism may be the factor which initiates the ultimate response by the thyroid to increase its activity. An intact pituitary would seem necessary for the response to occur.

That the level of cellular metabolism may influence the function of the thyroid in the opposite direction (*i.e.*, with decreased levels of serum protein-bound iodine) is shown by the findings of Goldberg and Chaikoff (17). They found that dinitrophenol administered to rats caused a progressive fall in the level of the circulating thyroid hormone without an evident increase in thyroid-stimulating hormone (TSH) production and without altered structure of the pituitary or thyroid. Our studies and those of Goldberg and Chaikoff's, therefore, suggest that the level of the circulating thyroid hormone is not the only factor controlling the production of TSH. If it were, it is difficult to see how either dinitrophenol or estrogen could alter the level of the SPI. The production of TSH should have promptly decreased with the estrogen-induced rise in SPI and increased with the dinitrophenol-induced fall in SPI. It is evident that the data cannot be entirely interpreted until the complete nature and function of TSH is understood. Greer (8) has recently suggested that more than one "thyrotropic" factor may be secreted by the pituitary.

SUMMARY

Our studies demonstrate that the concentration of serum precipitable iodine (SPI), a reliable index of the level of circulating thyroid hormone, increases in men or women during the administration of estrogen. A "leveling off" in the increments is usually observed by the third or fourth week. Normal function of the pituitary and thyroid are necessary for the reaction to occur. The SPI falls to control values when the estrogen is discontinued. Data are presented to indicate that the increments in SPI are due to an increase in activity of the thyroid, at least for a period of time, and not due to an altered rate of disposal of thyroid hormone. The studies suggest that increased elaboration of estrogen may be an important factor in the elevated concentrations of SPI seen in normal pregnancy. The administration of large amounts of estrogen appears to confer some increase in tolerance to increased amounts of circulating thyroid hormone. The underlying mechanism whereby the function of the thyroid is altered by estrogen is not entirely clear.

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DIAGNOSIS OF EQUIVOCAL HYPOTHYROIDISM, USING THYROTROPIC HORMONE (TSH)*†

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THYROTROPIC hormone (TSH) has proved to be a useful agent in differentiating primary thyroid myxedema from myxedema secondary to anterior pituitary insufficiency (1, 2, 3). In hypopituitarism the thyroid gland is dormant but responsive to stimulation, whereas in myxedema of thyroid origin there is no thyroid tissue to react. Using the uptake of radioactive iodine (I^{131}) by the thyroid gland as a measure of its function, TSH substantially augments I^{131} uptake above baseline levels in pituitary myxedema and in normal individuals, but fails to induce any significant increase in thyroid myxedema. It seemed logical, therefore, to apply this technique to the study of subjects with equivocal, borderline or doubtful states of thyroid failure, in order to ascertain the presence of responsive thyroid tissue. Lack of responsiveness to TSH should be correlated with a diagnosis of hypothyroidism. The differential diagnosis between hypometabolism, asthenia, debility, senility and hypothyroidism may occasionally be extremely difficult. Thyroid failure is often suspected in nephrosis, disorders of menstruation, infertility, habitual abortion, neurasthenia, psychoses, and edematous states. Equivocal laboratory studies may further confuse the issue and any new technique that may serve as a diagnostic adjunct is worthy of trial. This study concerns the effect of TSH upon the I^{131} uptake of a group of patients suspected of hypothyroidism, to determine whether their response could be correlated with the final diagnosis.

MATERIALS AND METHODS

Included in this study were 5 cases of post-thyroidectomy myxedema, 3 cretins, 14 examples of spontaneous myxedema, 12 patients with hypo-

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thyroidism or myxedema secondary to pituitary insufficiency, and 24 euthyroid subjects. These formed the background for an investigation of 17 patients suspected of hypothyroidism, who were diagnostic problems and who had been under observation for one year or more. The final diagnosis was ultimately established on the basis of repeated examinations, basal metabolism tests and serum cholesterol determinations before, during and following thyroid medication, and more than one therapeutic trial with desiccated thyroid in gradually ascending doses up to the tolerance of the patient. The response to TSH was not used to separate these 17 patients into 12 euthyroid and 5 hypothyroid individuals.

The technique of the TSH test was similar to that previously described (3). A tracer dose of 50 microcuries of carrier-free I^{131} was administered orally and the percentage in the thyroid gland twenty-four hours later was recorded by a Geiger counter. Approximately two weeks later this maneuver was repeated following three daily consecutive intramuscular injections of 20 to 30 milligrams of TSH. The magnitude of the percentage difference between the results of the two tracer studies was accepted, for the purpose of this work, as a measure of the functioning thyroid tissue.

RESULTS

A. *Myxedema*

The data in Table 1 demonstrate the clearcut difference in thyroid response between patients with primary thyroid myxedema and those with myxedema due to pituitary failure. TSH caused a mean increase of I^{131} uptake above baseline levels of 5 per cent in post-thyroidectomy myxedema and 0.2 per cent in cretinism and spontaneous thyroid myxedema, whereas in anterior pituitary insufficiency with secondary hypothyroidism the mean increase was 34 per cent. The test was accurate in reflecting the correct diagnosis in each of the 34 cases of hypothyroidism studied.

B. *Euthyroid subjects*

These individuals were divided into two groups, depending upon their initial I^{131} uptake. Those accumulating less than 15 per cent of the administered dose are shown in group A of Table 2 and patients accumulating 15 per cent or more are shown in group B. This division was necessary because, though most hypothyroid individuals accumulate little or no radioiodine, in rare instances the suspicion of hypothyroidism may be warranted, even in the presence of a normal I^{131} uptake. The mean increase produced by TSH in group A was 29 per cent and in group B, 18 per cent. There was a rough correlation, as shown in Figure 1, between the initial

TABLE 1. THE THYROID RESPONSE TO TSH IN 34 CASES OF THYROID FAILURE

Pt.	Diagnosis	BMR (%)	Serum chol. (mg./100 cc.)	I^{131} uptake (%)		
				Before TSH	After TSH	Diff. (%)
	A. Primary thyroid failure					
P.DeF.	Post-thyroidectomy myxedema	—	296	9	14	5
C.W.	Post-thyroidectomy myxedema	− 6	329	3	12	9
F.G.	Post-thyroidectomy myxedema	−14	359	9	16	7
J.R.	Post-thyroidectomy myxedema	—	—	4	15	11
M.W.	Post-thyroidectomy myxedema	−27	326	18	12	− 6
				Mean 8.6	*13.8*	*5.2 (Range −6 to 11)*
J.M.	Cretinism	−14	290	8	13	5
M.K.	Cretinism	—	224	6	6	0
R.W.	Cretinism	—	—	11	8	− 3
M.F.	Spontaneous myxedema	−50	420	3	3	0
G.H.	Spontaneous myxedema	−37	770	5	4	− 1
J.C.	Spontaneous myxedema	−44	495	7	5	− 2
H.M.	Spontaneous myxedema	−40	356	10	2	− 8
L.Z.	Spontaneous myxedema	−18	304	1	4	3
R.M.	Spontaneous myxedema	−25	463	5	3	− 2
M.S.	Spontaneous myxedema	—	300	7	12	5
A.M.	Spontaneous myxedema	− 2	276	7	11	4
A.S.	Spontaneous myxedema	−10	322	5	5	0
E.Ru.	Spontaneous myxedema	—	524	6	5	− 1
E.Re.	Spontaneous myxedema	−32	250	7	8	1
W.R.	Spontaneous myxedema	−43	289	9	12	3
M.S.	Spontaneous myxedema	—	—	9	7	− 2
J.McN.	Spontaneous myxedema	−36	331	7	7	0
				Mean 6.6	*6.8*	*0.2 (Range −8 to 5)*
	B. Thyroid failure secondary to pituitary insufficiency					
Y.L.	Sheehan's syndrome	13*	416	14	35	21
E.A.	Sheehan's syndrome	15	360	9	57	48
J.Z.	Acromegaly (inactive)	−47	228	17	57	40
T.D.	Chromophobe adenoma	− 4	311	8	40	32
V.Z.	Chromophobe adenoma	−20	291	15	54	39
E.S.	Chromophobe adenoma	−29	385	16	36	20
S.C.	Chromophobe adenoma	− 5	376	10	40	30
F.P.	Chromophobe adenoma	—	—	2	28	26
E.C.	Panhypopituitarism	−18	311	14	36	22
H.J.	Panhypopituitarism	—	323	15	59	44
A.A.	Panhypopituitarism	−27	295	10	47	37
F.L.	Panhypopituitarism	−38	—	11	60	49
				Mean 11.7	*45.7*	*34 (Range 20 to 49)*

* Congestive heart failure.

TABLE 2. THE THYROID RESPONSE TO TSH IN 24 EUTHYROID INDIVIDUALS

Pt.	Diagnosis	BMR (%)	Serum chol. (mg./ 100 cc.)	I^{131} uptake (%)		
				Before TSH	After TSH	Diff. (%)
	A. Patients with initial uptake below plus 15%					
A.M.	Schizophrenia	—	—	8	45	37
B.J.	Obesity; mastitis	− 8	—	14	46	32
M.N.	Normal	—	283	11	26	15
R.G.	Normal	—	342	10	35	25
E.A.	Normal	—	—	13	42	29
W.McC.	Normal	—	—	10	46	36
				—	—	—
				Mean 11	*40*	*29 (Range 15 to 37)*
	B. Patients with initial uptake 15% or above					
E.D.	Irreg. menses	−15	295	15	40	25
D.M.	Irreg. menses	− 9	185	26	41	15
H.V.	Obesity	− 6	250	22	52	30
B.M.	Obesity	− 6	274	27	48	21
J.Y.	Obesity	−19	—	29	36	9
M.N.	Exophthalmos	− 6	523	18	45	27
A.F.	Infertility	−27	255	23	39	16
M.G.	Infertility	+ 1	221	20	38	18
E.W.	Menopause	+ 1	200	18	31	13
W.P.	Schizophrenia	—	—	21	33	12
D.G.	Schizophrenia	—	—	19	54	35
C.G.	Schizophrenia	—	—	26	34	8
H.K.	Schizophrenia	—	—	21	27	6
B.D.	Normal	+ 1	150	29	41	12
G.W.	Normal	−10	206	21	43	22
J.F.	Normal	—	241	30	38	8
M.L.	Normal	−19	150	16	43	27
K.W.	Normal	−18	248	16	36	20
				—	—	—
				Mean 22	*39.9*	*17.9 (Range 6 to 35)*

uptake and the increase produced by TSH, lower initial values being generally associated with larger responses to TSH.

C. *Equivocal hypothyroidism*

In 12 of the 17 cases of equivocal hypothyroidism normal thyroid function was eventually demonstrated. The TSH response agreed with the diagnosis in 11 of the 12 cases; the rise in I^{131} uptake varied from 14 to 40 per cent, as shown in Table 3. In 1 case (K.B.), in which no increase occurred, the initial uptake was normal. This patient had been repeatedly referred to the endocrine clinic during a three-year period with the diagnosis of probable myxedema and each time, after appropriate study and observation, normal thyroid function was proved. Her failure to demonstrate an increased radioiodine uptake following TSH stimulation may either represent a shortcoming of the test or may signify that her thyroid gland was already functioning at a maximum level and could not be stimulated further. In Table 2 we find that 4 of the euthyroid individuals in group B (J.Y., J.F., C.G. and H.K.), whose initial radioiodine uptakes were within the normal range, showed a minimal response to TSH, indicating that their ability to accumulate radioiodine was at or near maximum for this dose level of TSH.

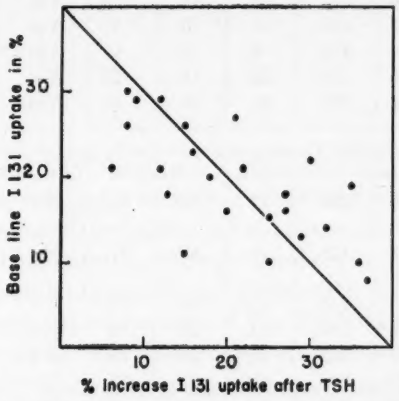

FIG. 1. The effect of the initial I^{131} uptake on the thyroid response to TSH in euthyroid subjects.

D. *Reproducibility of the TSH response*

The TSH test was repeated in 3 patients after an interval of three to six months, as shown in Table 4. No significant differences between the results of the first and second tests were observed.

TABLE 3. THE THYROID RESPONSE TO TSH IN 17 PATIENTS WITH SUSPECTED OR EQUIVOCAL HYPOTHYROIDISM

Pt.	Age	Sex	BMR %	Serum chol. (mg./ 100 cc.)	I^{131} uptake (%)			Response to thyr. therapy	Final diagnosis
					Before TSH	After TSH	Diff. (%)		
A. Suspected hypothyroidism—finally proved									
A.W.	30	F	+ 5	177	8	16	8	Yes	Hypothyroid
M.W.	44	F	−27	145	4	5	1	Yes	Hypothyroid
A.C.	70	F	−24	474	4	4	0	Yes	Hypothyroid
M.S.	55	F	−25	315	18	16	−2	Yes	Hypothyroid
S.D.	54	F	−21	230	11	14	3	Yes	Hypothyroid
B. Suspected hypothyroidism—finally proved euthyroid									
B.M.	46	F	+ 6	282	12	32	20	No	Obesity; menopause; psychoneur.
D.W.	22	F	−18	128	18	45	27	No	Nontoxic diffuse goiter
K.W.	26	M	−18	248	15	36	21	No	No disease
P.L.	41	F	+10*	187	11	25	14	No	Obesity; menopause
M.N.	45	F	− 1	342	11	26	15	No	No disease
P.J.	51	M	−28	663	10	33	23	No	Nephrosis
E.J.	53	F	−19	262	21	58	37	No	Menopause
M.R.		F	−11	343	23	42	19	No	No disease
K.B.	55	F	+ 6	247	22	22	0	No	Psychoneur.
S.R.	32	F	−20	300	9†	49	40	No	Psychoneur.
I.E.	66	F	−24	260	6†	33	27	No	Invol. melancholia
R.B.	41	F	−39	254	13	36	23	No	Obesity; menopause

* BMR unreliable.

† While receiving thyroid, 0.13 Gm. daily.

E. *The effect of thyroid medication*

Thyroid medication markedly inhibits and, in sufficiently large doses, may completely suppress the uptake of radioiodine by the thyroid gland (4). That exogenous TSH can overcome this effect in the presence of potentially active thyroid tissue is demonstrated in Table 5. Two euthyroid patients, originally suspected of being hypothyroid, were studied while receiving 0.13 Gm. of desiccated thyroid daily and each showed a substantial increase of I^{131} after TSH.

DISCUSSION

Thyroid responsiveness to TSH was correlated with a euthyroid state in 11 of 12 patients studied. Lack of response to TSH was found in all of

TABLE 4. THE REPRODUCIBILITY OF THE TSH TEST

Diagnosis	Interval between tests (mos.)	I^{131} uptake (%)		
		Before TSH	After TSH	Diff. (%)
Euthyroidism	6	I 12.5	36.4	23.9
		II 9	36	27
Hypopituitarism	3	I 8	40	32
		II 3	51	48
Hypopituitarism	3	I 17	57	40
		II 22	57	35

5 patients in whom a final diagnosis of hypothyroidism was established. Thus, of 17 patients originally suspected of being hypothyroid, the result of the TSH test was correlated with the final diagnosis in 16. These 17 subjects had presented diagnostic problems resolved only after one year or more of repeated examinations, several therapeutic trials with desiccated thyroid and numerous determinations of the BMR and serum cholesterol. A case typical of the group will illustrate some of the problems encountered.

Mrs. S.R., 32 years old, complained of extreme fatigue, lethargy, constipation, sensitivity to cold, weight gain, dry hair and brittle nails. Physical examination revealed hypotension (B.P. 100/66), some dryness of the hair and skin, and pallor. Laboratory studies gave the following values: BMR −19 per cent, serum cholesterol 300 mg./100 cc., protein-bound iodine 3.4 micrograms/100 cc., and hemoglobin 11.8 Gm. A therapeutic trial seemed warranted, and 0.03 Gm. of thyroid was prescribed daily. The patient stated that she felt more energetic, less sensitive to cold and less constipated. Within three weeks this "good effect wore off" and subsequent increases up to 0.3 Gm. of thyroid were ineffective in preventing the return of all her symptoms. After eight weeks of thyroid therapy her BMR was still −20 per cent. While receiving 0.13 Gm. of thyroid her I^{131} uptake was 9 per cent; after TSH it rose to 49 per cent. Euthyroidism was confirmed by her sub-

TABLE 5. THE RESPONSE TO TSH DURING THYROID MEDICATION IN SUSPECTED HYPOTHYROIDISM

Diagnosis	BMR (%)	Serum chol. (mg./100 cc.)	Serum PBI (μg./100 cc.)	Thyroid medication (Gm./day)	I^{131} uptake (%)		
					Before TSH	After TSH	Diff. (%)
Anxiety neurosis	−19	300	3.4	0.13	9	49	40
Involutional melancholia	−24	260	—	0.13	6	33	27

sequent clinical course and thyroid therapy was omitted without incident in the ensuing six months.

This patient demonstrates the usefulness of the TSH tests and shows, in addition, as pointed out by Stanley and Astwood (4), that the potentially functional thyroid gland responds to TSH even during thyroid medication. It is, therefore, unnecessary to omit thyroid and await the termination of exogenously inhibited thyroid activity (Farquharson effect) when evaluating thyroid function with TSH. The value of the TSH response may be further enhanced by measuring other indices of thyroid function concomitantly with the radioiodine uptake. Thus the blood protein-bound iodine (PBI) level, may add useful diagnostic information, as illustrated in Patient F.P., group B, Table 1. Following TSH administration, her PBI rose from 3.5 micrograms to 13.2 micrograms per 100 cc. The TSH response was elicited in a patient suffering from severe malnutrition: W.K., a debilitated male with a six-year history of sprue, showed a rise from a baseline I^{131} uptake of 4 per cent (oral and intravenous I^{131}) to 26 per cent after TSH administration. This response was probably due to hypopituitarism induced by inanition.

The TSH test may confuse the issue when thyroid failure is secondary to adenohypophyseal insufficiency. The augmented uptake produced by TSH may be similar in euthyroid subjects with initial low uptakes and in patients with pituitary hypothyroidism. Thus, a positive response to TSH is less clearcut in its implications than is a negative response, and may warrant further study of pituitary function.

The ability of TSH to cause an increase in iodine accumulation by the thyroid may not, on theoretical grounds, eliminate the possibility of clinical hypothyroidism, even in the absence of pituitary insufficiency. This may be particularly true in post-thyroidectomy hypothyroidism, in which a remnant of tissue, inadequate to synthesize sufficient thyroxine, may still demonstrate responsiveness to TSH. In Table 1, it is evident that the post-thyroidectomy myxedema cases did indeed exhibit a somewhat greater response than did patients with cretinism and spontaneous myxedema, though the difference was small.

Levy, Palmer and Jefferies recently reported a similar study (5) which has been published only in abstract form at the time of this writing. Their technique differed in some details but their conclusions were in agreement with ours.

In this small series of patients the thyroid response to TSH proved to be a useful diagnostic adjunct in the study of suspected hypothyroidism. Its value was further enhanced by its accuracy in patients who were receiving thyroid medication during the performance of the test.

SUMMARY

Thyrotropic hormone (TSH) induces a marked rise of radioiodine (I^{131}) uptake by the thyroid gland in euthyroidism and pituitary hypothyroidism but not in primary thyroid myxedema. It was used, therefore, in the study of a group of subjects with suspected or equivocal hypothyroidism, to determine whether the thyroid response to TSH could be correlated with the final diagnosis. The TSH response was an accurate index in 16 of 17 patients studied. Thyroid medication did not inhibit the action of TSH. Repetition after as long as six months demonstrated that the results were reproducible. Since the augmentation of I^{131} uptake may be similar in normal subjects and in pituitary hypothyroidism, the latter diagnosis must be considered as a possible source of diagnostic error. A positive response is, therefore, in these rare instances, of less diagnostic value than a negative response.

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ON THE MODE OF ACTION OF IODOTHIOURACIL

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SINCE the synthesis of 5-halogeno-2-thiouracils by Barrett, Goodman, and Dittmer (1), the claim has been advanced that 5-iodo-2-thiouracil, originally selected for further study partly because of its goitrogenic activity (2), "possesses a unique antithyroid action," and "it is suggested that the unique effect of the iodo derivative is due to the entire molecule rather than to any particular moiety" (3). The claim for uniqueness has been based on observations which "indicate that in the animals tested 5-iodo-2-thiouracil is a potent antithyroid agent but possesses little if any goitrogenicity when compared with non-iodinated thiouracil" (4). The precise significance of the qualifying phrase in the last quotation is not altogether clear. Although it must be evident that thought-control is as out of place in endocrinology as humor in a dictionary (5), it would seem a reasonable corollary that scepticism must flourish. Thus it is important that new approaches to antithyroid agents be made and that claims for uniqueness be greeted critically.

In the present instance the statement made by Gassner *et al.* (4), Williams *et al.* (6), and Catz *et al.* (7), to the effect that iodination of thiouracil might increase the concentration of the compound in the thyroid due to the great affinity of the gland for iodine, arouses scepticism, for Leblond and Sue (8) have shown in regard to other iodine-bearing compounds that only after breakdown and reduction to iodide does concentration by the thyroid gland occur. This clearly does not negate the possibility that iodination of thiouracil will increase concentration by the thyroid gland, but would appear to be evidence against the likelihood.

Scepticism would appear appropriately to be aroused by the claim for a unique mode of action of iodothiouracil since it is a combination of two substances, each of which modifies the ill-understood process of thyroid hormone formation in a manner incompletely defined. Further, it is known from the studies of Astwood (9) that substitutions of polar groups in position 5 and 6 in the thiouracil molecule result in great alterations in antithyroid activity, even when the substituent has no known effect on

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thyroid function. Thus, 6-aminothiouracil, 6-carboxythiouracil, and 5-cyanothiouracil are nearly devoid of antithyroid activity. In some respects 5-iodothiouracil may be held a comparable substance. The problem in regard to 5-iodothiouracil is to assay the effects of this substance itself and to determine those of its breakdown products and the relative significance of each. The present work does not provide answers to this problem, but it does challenge the concepts of iodothiouracil as an agent which is antithyroid but not significantly goitrogenic and as having its effects by blocking thyrotropin action without increasing its elaboration. The process of thyroid hormone formation is incompletely understood and the manner in which it is altered by iodine, especially in the instance of Graves' disease, is obscure. Although the hypothesis of two modes of action of thiouracil, either as a reducing agent or as a peroxidase inhibitor, is plausible, the actual mode of action is not yet proved. The law of parsimony in logic requires that the simplest explanation is to be held the most likely, and in the present instance it appears to enjoin against the too-ready acceptance of a unique manner of action for a substance composed of two moieties, each of which impinges on an obscure process in an unknown way.

METHODS

Male rats of the Sprague-Dawley strain, 21 days old, were fed diets either of Purina Mink Chow or of low-iodine content, into which 6-propylthiouracil, Priodax (iodoalphionic acid) tablets, or 5-iodo-2-thiouracil was mixed after being ground to a fine powder in a mortar. The rats were weighed at the beginning and end of ten-day periods during which they were fed experimental diets, the amount of food consumed being weighed.

The low-iodine diet was composed of cracked wheat 660 Gm., casein 150 Gm., Klim 150 Gm., yeast 25 Gm., $CaCO_3$ 10 Gm., and NaCl 5 Gm.; to each kilogram of diet 1.0 ml. of cod liver oil was added.

After ten days the rats were killed with chloroform or were bled to death under ether anesthesia. The thyroid glands were dissected and weighed on a Roller-Smith torsion balance. Some glands were fixed in 10 per cent formalin, others were placed in 1.0 ml. of chloric acid for iodine determination by a modification of the method of Zak *et al.* (10).

When thyroid : serum iodide ratios were determined the rats were injected with 10 mg. of propylthiouracil and one-half hour later with 10 microcuries of I^{131}; forty-five minutes later the rats were anesthetized with ether and bled. Radioactivity per unit of weight of serum and of thyroid were compared, counting being performed at 1-ml. volume, determining γ radiation.

The iodide-concentrating mechanism of the thyroid gland was inhibited by injecting 1 mg. of KSCN subcutaneously one hour prior to killing the rats.

RESULTS

Figure 1 shows the effects on thyroid weight of 1 per cent and 3 per cent concentration of 5-iodo-2-thiouracil in Purina Mink Chow fed for ten days to normal 21-day-old rats. The figure shows 4 control rats to have thyroid

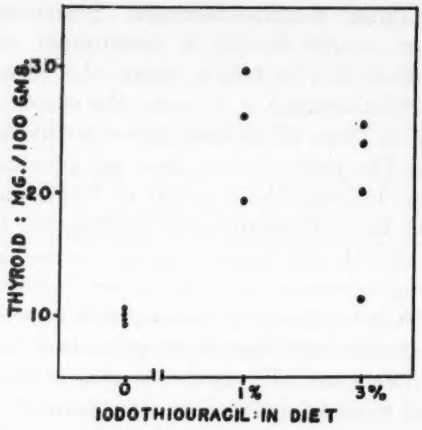

FIG. 1. The effect of addition of iodothiouracil in 1 and 3 per cent concentrations in Mink Chow is to increase thyroid weight to two or three times the normal value.

glands weighing 10 mg. per 100 grams of body weight. In contrast, rats fed diets with 1 per cent iodothiouracil had thyroid glands weighing two to three times the control value. Average values in the group receiving diets with 3 per cent iodothiouracil were slightly lower, but again thyroid sizes as much as 2.5 times the control values were found. The adrenal glands of the iodothiouracil-fed rats were not increased in weight; but gain in body weight did not occur in rats fed the greater concentration of drug.

In Figure 2 are found the results of a similar experiment in which increasing concentrations of iodothiouracil were added to a diet low in iodine. At 1 per cent dietary concentration, glands 80 per cent heavier than glands from rats fed no iodothiouracil were found, and when increasing doses of iodothiouracil versus thyroid weight were plotted on a semilogarithmic scale a straight-line relationship was observed.

The glands from the experiments just described were noted to be red and vascular at the time of dissection and no gross difference from glands of similar enlargement due to propylthiouracil feeding could be observed. The foregoing observations indicated that iodothiouracil is goitrogenic to a significant degree and the gross finding of reddened vascular thyroid glands suggested thyrotropic hormone stimulation. Formalin-fixed material was sectioned and stained and iodothiouracil was found to have induced marked evidence of stimulation, judged by increase in cell height and loss of colloid from the follicles.

It is known that Priodax, like iodothiouracil, increases the "protein-bound iodine levels" of the blood in man (11, 12) and it was assumed that

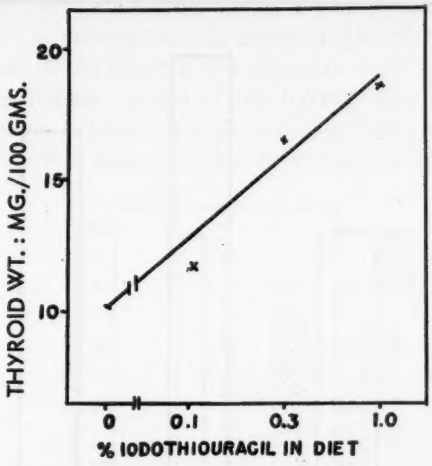

FIG. 2. The addition of increasing concentrations of iodothiouracil to a low-iodine diet causes increasing thyroid weight—represented as a straight-line relationship on a semilogarithmic plot.

similar effects might occur in rats, although no evidence was at hand to ensure the validity of the assumption. Nonetheless, Priodax, which contains 50 per cent iodine, was fed in 0.2 per cent dietary concentration to rats over ten-day periods and the thyroid gland weights resulting from this regimen were compared with those resulting from 0.2 per cent iodothiouracil concentration in the diet (Mink Chow). Figure 3 shows that iodothiouracil was goitrogenic, whereas the diet containing Priodax resulted in no change in thyroid size from controls shown in Figure 1. Figure 3 also depicts the effect on thyroid size of ten days' feeding of 0.003 per cent dietary concentration of propylthiouracil and further indicates that propylthiouracil was slightly less goitrogenic when Priodax was mixed into the diet in 0.2 per cent concentration. On the other hand, 0.2 per cent dietary iodothiouracil, containing the same amount of iodine, resulted in no modification of the goitrogenicity of propylthiouracil.

From the work of Astwood (13) it is known that chronic thiouracil administration results in a fall in iodine concentration in the thyroid gland and a markedly increased avidity for iodine. Subsequently in his laboratory it was shown (14) that it is the iodide ion which is trapped under this circumstance and that with hyperplasia and hypertrophy the concentration gradient for iodide between serum and thyroid may be increased tenfold and that the administration of thiocyanate inhibits the mechanism for trapping iodide. It is therefore known that a greatly increased iodide space results from thyrotropic stimulation occurring in the course of antithyroid

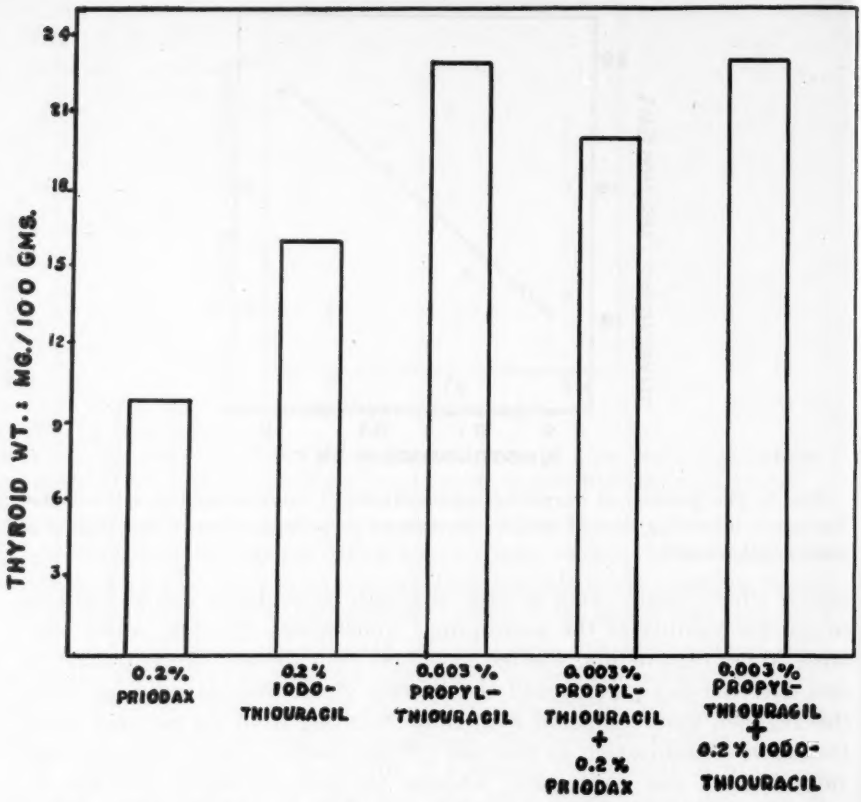

FIG. 3. Priodax in 0.2 per cent dietary concentration results in no change in thyroid weight compared with the normal (see Figure 1). Iodothiouracil and propylthiouracil each cause thyroid enlargement. The goitrogenicity of propylthiouracil is slightly modified by Priodax but not by iodothiouracil.

drug administration. It was also shown that the concentration gradient was constant over a wide range of iodine dosage but that the mechanism could be flooded, so that with very large doses of iodine the thyroid: serum iodide ratio fell from values as high as 300:1 toward 1:1. In the event of a "flooded" iodide-concentrating mechanism it is not possible to demonstrate further uptake of iodide, either by radioactive measurement or by chemical determination. However, thiocyanate discharges the iodide in large part, so that comparison of the iodine content of animals receiving iodothiouracil with that of animals similarly treated, but with the addition of thiocyanate prior to death, should allow the iodide space to be calculated by difference.

Figure 4 shows the thyroid size, the total iodine content and the small iodide space found in normal rats; in contrast, the effects of feeding 1 per cent iodothiouracil in the diet for ten days are to be noted. Thyroid size was doubled. The iodine content of the thyroid gland was only slightly diminished. However, in those animals receiving thiocyanate prior to death the iodine content was greatly lowered, indicating an iodide space in-

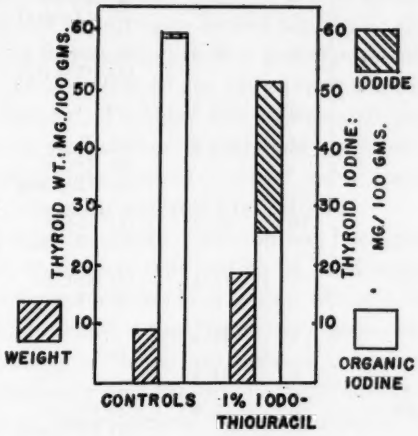

FIG. 4. Iodothiouracil not only increases thyroid weight but greatly increases the iodide space. The iodide space was obtained by difference between the total iodine content of rats receiving iodothiouracil only, and that of rats into which 1 mg. of KSCN was injected subcutaneously one hour before death. The latter give values represented as organic iodine.

creased many fold over that of normal rats. Determination of the thyroid: serum iodide gradient revealed values in the range of 12:1 in contrast to control values ranging from 19:1 to 32:1 for rats fed a diet low in iodine. From the size of the iodide space and the concentration gradient it may be calculated that the serum iodide value from iodothiouracil administration is sufficiently high to cause "flooding" of the iodide-concentrating mechanism (14).

Data from which the figures were drawn are presented in Table 1.

DISCUSSION

The experiments reported in this study indicated that 5-iodo-2-thiouracil caused enlargement of the thyroid gland to two or three times the normal size after a ten-day feeding period. *In situ* these glands were red and vascular, and histologic evidence of stimulation was present. When added to propylthiouracil-containing diets, iodothiouracil caused no diminution of

TABLE 1

Medication* mixed with diet	No. of rats	Average thyroid wt. (mg./100 Gm.)	Medication injected and No. of rats†	γI_2 per 100 mg. thyroid (av.)	T/S iodide ratio (av.)
Low iodine diet					
0.1% IT	6	12.6	(2) KSCN (3) PT+RaI	27.5 21.7	9.06
0.3% IT	6	16.5	(3) KSCN (3) PT+RaI	27.9 31.2	23.2
1.0% IT	5	18.8		26.9	
1.0% IT	16	17.8	(6) No inj. (6) KSCN (4) PT+RaI	38.8 21.4	12.7
1.0% IT	10	18.4	(5) KSCN (5) PT+RaI	26.4 52.2	12.6
0.001% PT+.7% KI	5	17.1	(3) KSCN (2) PT+RaI	9.55 9.75	6.74
0.5% PT	6	28.4		2.1	
0.5% PT+0.7% KI	6	24.4		24.3	
0.002% PT+.7% KI	5	17.4	(2) KSCN (3) PT+RaI	13.1 14.8	3.9
Controls	6	9.9	(3) KSCN (3) PT+RaI	56.9 56.6	33.0
Controls	8	11.8	(3) No Inj. (4) PT+RaI	29.7	19.3
Controls	8	8.85	(4) No inj. (4) PT+RaI	59.5	31.7
Purina Mink Chow diet					
Controls	4	10.2			
1% IT	3	25.2			
3% IT	4	20.1			
0.2% Priodax	4	9.8			
0.2% IT	4	15.9			
0.003% PT	6	23.0			
0.003% PT+0.2% Priodax	6	20.2			
0.003% PT+0.2% IT	6	23.1			

* IT = Iodothiouracil
PT = Propylthiouracil
KI = Potassium iodide
KSCN = Potassium thiocyanate
RaI = Radioactive iodine
T/S = Thyroid to serum
† Number of rats shown in parentheses.

the goitrogenicity of propylthiouracil, as has been observed by McGinty (15) with iodine, and here with Priodax. Iodothiouracil administration caused a great increase in the iodide space of the thyroid glands of rats, although the amount of iodide in the serum was regularly so great as to flood the iodide-trapping mechanism of the thyroid glands. The effects on vascularity, the production of goiter, and the great increase in the iodide space of the thyroid gland are effects now generally accepted to be those of elaboration of thyrotropin by the pituitary; and the results of this study indicate that iodothiouracil is a goitrogenic agent which releases such a large amount of iodide to the blood that the mechanism for iodide concentration is flooded. Further, the evidence presented, although indirect, favors the viewpoint that thyrotropin elaboration by the pituitary is increased following the administration of iodothiouracil to rats. This is in support of the data of Albert and Ford (16).

It is submitted that too great attention has been paid to administering iodothiouracil and thiouracil plus iodine in equimolar concentrations in the evaluation of the general mode of action of the former. An example of the confusion which could arise from such considerations being overweighed in formulating a theory of mode of action can be made using methylmercaptoimidazole and thiouracil. Both substances in man reduce metabolism and can cause severe hypothyroidism. However, thiouracil is only 1.0 per cent as active as mercaptoimidazole (17) on a weight basis, and, if this be made the chief consideration and thiouracil be administered in amounts equimolar to methylmercaptoimidazole, it could be concluded that thiouracil is not goitrogenic. In rats, on the other hand, methylmercaptoimidazole is only twice as active as thiouracil so that comparisons of a similar sort would result in the demonstration that each agent is goitrogenic in this species.

In regard to the importance of the iodine portion of iodothiouracil, Figures 1 and 2 indicate that it is not a dominant factor in view of the studies of Remington and Roe (18), which showed that increasing the dietary concentration of iodine resulted in diminution of the thyroid weight of rats. On the other hand, clinical studies in man suggest that the role of iodine is dominant in the treatment of hyperthyroidism with iodothiouracil (6, 7).

The finding in this study that iodothiouracil is goitrogenic in rats and the inference from clinical studies that iodothiouracil in hyperthyroidism acts principally by virtue of its iodine content may appear contradictory. However, it is perhaps significant that in the rat a condition of euthyroidism initially exists and the thyroid enlargement which results from iodothiouracil administration is *per se* evidence of hypothyroidism. This

effect has not been observed from iodine. In man, however, reduction of hyperthyroidism to euthyroidism is attempted, an effect which can result from iodine administration as well as from thiouracil and its derivatives. An example of the difference between reduction of a euthyroid state to hypothyroidism and of hyperthyroidism to euthyroidism is found in the consideration of goiter and thiouracil. Chronic thiouracil administration is generally recognized to induce goiter in the normal rat, man, and other species. In human hyperthyroidism, however, chronic administration of thiouracil results in a gradual shrinkage of thyroid size (19) unless hypothyroidism is induced; in this event rapid enlargement of the thyroid gland occurs. It is therefore apparent that the conditions of the experiment determine whether such an antithyroid drug is antigoitrogenic or goitrogenic.

SUMMARY

In rats the chronic administration of iodothiouracil results in significant goiter, increased thyroid vascularity, increased cell height, discharge of colloid, and a many-fold increase in the iodide space of the thyroid gland. At all concentrations used, flooding of the iodide-trapping mechanism was found. These observations are interpreted as indicating that in the rat iodothiouracil acts to interfere with thyroid hormone synthesis, to increase thyrotropic hormone elaboration and thus to result in goiter. The concept that iodothiouracil has a unique mode of action is not supported by this study, nor is it clear whether the effects of iodothiouracil administration are due to the substance itself or to its metabolites.

Acknowledgment

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PENETHAMATE HYDRIODIDE (NEO-PENIL): INFLUENCE ON BLOOD PROTEIN-BOUND IODINE LEVELS

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BLOOD protein-bound iodine (PBI) determinations have become an important tool in the estimation of thyroid function. Factitious elevation of the PBI is caused by drugs and diagnostic agents which contain iodine. It appeared of interest to determine whether a similar effect was exerted by penicillin G diethylaminoethyl ester hydriodide (Penethamate Hydriodide or Neo-Penil®).

Four individuals who were clinically euthyroid were studied. One patient with lung abscess received 500,000 units of Neo-Penil intramuscularly every six hours for nineteen days. A second patient with lung abscess was given a similar dosage for four days. Two healthy physicians were each given a single injection of 500,000 units intramuscularly. Blood protein-bound iodine was determined by the method of Connors (1). The normal range is considered to be from 3.5 to 6.7 micrograms per 100 cc. of blood.

The results are shown in Table 1. In 2 normal subjects, single doses of 500,000 units elevated the PBI concentrations to the hyperthyroid range twenty-four hours after the drug was administered. The levels returned to the normal range within the next forty-eight hours in both instances. PBI levels greater than 30 micrograms per 100 cc. of blood were found in both patients with lung abscess receiving 2,000,000 units of Neo-Penil daily. Levels determined one week after discontinuation of Neo-Penil therapy were within the normal range.

DISCUSSION

Spurious elevation of blood protein-bound iodine levels (not due to hyperthyroidism) is caused by a variety of medications and diagnostic procedures. Contrast-medium roentgenographic studies, such as angiocardiography, cholecystography, urography, bronchography or myelography, employ iodine-containing compounds; even the barium mixtures

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TABLE 1. EFFECT OF PENETHAMATE HYDRIODIDE (NEO-PENIL®) ON BLOOD PROTEIN-BOUND IODINE LEVELS

Patient	Dose of Penethamate	Before medication	Blood protein-bound iodine conc. (mcgm. per 100 cc.)		
			24 hrs. after last dose	72 hrs. after last dose	168 hrs. after last dose
N.R.	500,000 U. q. 6 h. for 4 days	—	Over 30	—	4.0
W.A.	500,000 U. q. 6 h. for 19 days	—	Over 30	—	6.6
G.T.	500,000 U. (single dose)	5.6	8.4	4.2	—
L.J.	500,000 U. (single dose)	7.0	12.1	3.9	—

used in gastrointestinal studies may contain enough iodine to affect blood iodine levels (2). Drugs which influence the concentration of protein-bound iodine include the iodides, iodothiouracil, and iodine-containing amebicides.

A new iodine-containing penicillin preparation has been shown to cause blood protein-bound iodine levels in the thyrotoxic range, and must be added to the foregoing list. The effect is a transitory one, even after prolonged administration of large doses, in contrast to the elevations produced by roentgenographic use of iodine compounds, which may last for months or years.

SUMMARY

Penethamate Hydriodide (Neo-Penil®), an iodinated ester of penicillin, has been shown to cause high blood protein-bound iodine levels. Administration of this drug will temporarily invalidate the use of the PBI determination as a test of thyroid function.

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Letters to the Editor

A NOTE OF CAUTION ON THE HEMOLYTIC PROPERTIES OF WATER-SOLUBLE ANDROGENS*

To the Editor:

There are now many occasions on which one would like to administer large quantities of steroid by the intravenous route. This note is intended merely as a word of caution, pointing out a property of water-soluble testosterone derivatives, which is not generally recognized and which makes it imperative that certain of these agents not be administered parenterally.

In the course of studies on steroid therapy of advanced cancer, it was deemed advantageous to administer by continuous intravenous injection fairly large quantities of a water-soluble derivative of testosterone (testosterone 17-β,diethyl-amino-ethyl-carbonate hydrochloride). It was found that such administration led to severe hemoglobinuria. Fortunately, in this patient, there were no untoward consequences. Further investigation was undertaken, and it was found both *in vivo* and *in vitro* that testosterone 17-β,diethyl-amino-ethyl-carbonate hydrochloride and sodium testosterone sulfate are both extremely potent agents in producing hemolysis.

Experiments in both rats and dogs confirmed the finding that by both intravenous and subcutaneous injection, it is possible to kill animals readily with either of these agents and that the animals die with extensive intravascular hemolysis. This is not due to failure of the body to hydrolyze testosterone 17-β,diethyl-amino-ethyl-carbonate hydrochloride, since the administration was followed by an outpouring of 17-ketosteroid, presumably from the administered material.

The hemolytic and toxic properties in the rat and dog have been confirmed in the Ciba Laboratories for testosterone 17-β,diethyl-amino-ethyl-carbonate hydrochloride.

This note is intended as a caution to people interested in administering such compounds by the intravenous route particularly. It is not intended as an extensive report of research results along these lines, but rather as an observation which will serve to bring this distressing property of these

* This investigation was supported by an Institutional Research Grant from the American Cancer Society, Inc.

agents to wider recognition. It should be pointed out that possibly other water-soluble derivatives of steroids also may display this property.

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HILAR-CELL PROLIFERATION IN THE ADRENOGENITAL SYNDROME

TO THE EDITOR:

In a recent paper (1) the occurrence of nodules of cells in the hilar regions of the testes of males with the familial congenital adrenohyperplastic form of the adrenogenital syndrome was discussed. Evidence indicating that these nodules are composed of Leydig rather than of ectopic adrenal cells was presented, and their occurrence was felt to support the theory that the basic defect in the disease lies in the pituitary rather than in the adrenal. At that time, no similar nodules were found in the routine sections of ovary available from 5 newborn female infants with this disease; and of 5 reports which might possibly concern such a lesion, none was considered convincing. It was therefore suggested that "a distinct difference between males and females with this disease may exist in this regard."

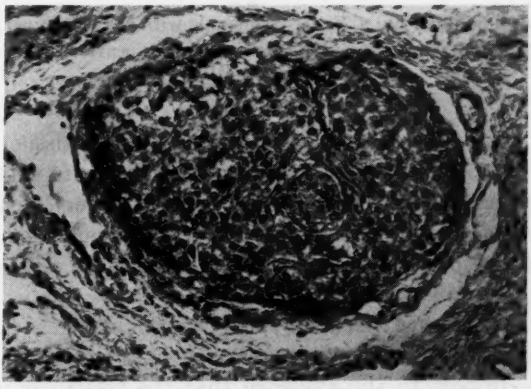

FIG. 1. Hilar-cell nodule from a 1-month-old female with adrenal hyperplasia and pseudohermaphrodism. Note the lack of zonation or radial cord-pattern, as seen in ectopic adrenal nodules, and the small central nerve (×160).

Recently, in a study of material from 4 other affected females, collections of cells, arranged around small nerves, were seen in the ovarian hilar regions of 2 of the patients. The appearance of these nodules, with their lack of cord-pattern, is typical of hilar-cell accumulations (Figs. 1 and 2)

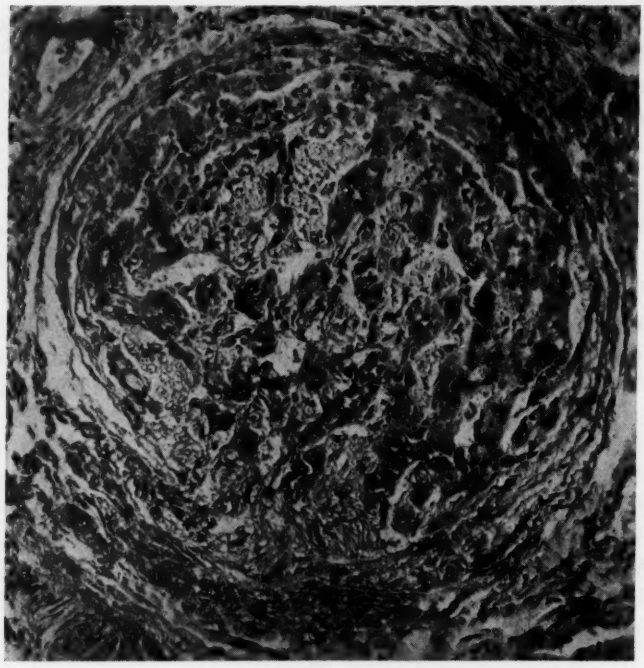

FIG. 2. Hilar-cell nodule from a 7-year-old female with adrenal hyperplasia and pseudohermaphrodism. Note the many small nerve bundles scattered through the nodule (×160).

and different from that of nodules of ectopic adrenal tissue (hyperplastic Marchand's rests), which are also seen in many patients with this disease. One of these patients was aged 1 month, and the other 7 years; in neither instance were the hilar cell nodules seen on gross examination, so that there was little evidence of a tendency to enlargement with age, as in the case of the testicular nodules.

A degree of difference between males and females with this disease apparently does exist, but the occurrence of hilar-cell nodules in affected females lends support to the evidence that the testicular nodules in affected males are composed of Leydig cells, and indirectly to the theory that abnormal pituitary gonadotropic secretory activity is a basic feature

of this condition. Since a physiologic difference between intertubular and hilar Leydig cells may exist, it would perhaps be better to speak of the nodules in both sexes as composed of hilar cells.

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Teaching Clinic

HYPOGLYCEMIA

INSTRUCTOR: Spontaneous hypoglycemia is a condition which reflects a disturbance in the complicated machinery of carbohydrate metabolism. It reveals itself in a protean set of signs and symptoms which may lead the physician into various errors of diagnosis. Indeed, the chief complaints may include sweating, nausea, weakness, dizziness, palpitation, abdominal distress, restlessness, nerve palsies, muscle spasm, convulsions and even behavioral and emotional disturbances. A patient who presents any part of such a picture of autonomic or central nervous system involvement deserves a blood sugar determination in the study of his illness.

The cases presented here depict some of the problems in determining the etiology of hypoglycemia, and emphasize the methods which we consider useful in reaching a diagnosis. The full differential diagnosis of all causes of hypoglycemia and the many disorders associated with convulsions or other nervous symptoms will not be discussed. The former topic has been ably presented by Conn (1); the problems of the nervous symptoms would require a textbook. Here the limited topic of distinguishing between functional hypoglycemia and islet-cell tumor will be reviewed, with a passing digression on the differentiation or relation between such nervous symptoms and those of epilepsy.

The first patient to be considered is **Mrs. H.L.**, a 32-year-old white woman, who was admitted to the Hospital of the University of Pennsylvania on August 31, 1953. She had been in good health until January 1953, when she began to have episodes of weakness, dizziness and headache. These symptoms occurred about 3:00 P.M., and were worse when she was at work as a hairdresser. She also noted numbness about her face and extremities. These episodes, which she experienced almost daily, would last about two hours and were relieved either spontaneously or by drinking a glass of milk. In May 1953 the patient's husband began to have difficulty awakening her each morning. He had to walk her about to arouse her, and he noted that she was sweating at these times. When she did awaken, she was somewhat weak, but she felt well the rest of the day. Neither she nor her husband was able to give a definite picture of relief of her symptoms by food. Moreover, the patient was unable to remember the episodes well.

Four weeks before her admission to this hospital, she was studied elsewhere for encephalitis, since one physician had noted a white blood cell count of 19,000 per cubic millimeter. X-ray examination of the skull, and cerebrospinal fluid studies showed nothing abnormal, however, and she was discharged with a tentative diagnosis of psychoneurosis. A few days later she had a generalized convulsion for which she was admitted to the neurosurgical service of this hospital (Dr. F. C. Grant).

Physical examination revealed a somewhat obese woman who responded intelligently to questions. She did not show any abnormal findings. The results of a complete neurologic examination were entirely normal. The tentative diagnosis before laboratory studies were performed was idiopathic epilepsy.

On the morning after admission, she was stuporous and the fasting blood sugar level at this time was 20 mg. per 100 ml. Her stupor was relieved by orange juice.

With this new fact at hand, what is your diagnosis and what further studies are needed?

STUDENT:[1] Hypoglycemia would explain all her symptoms. The cause or type of hypoglycemia requires further study. The commonest causes are functional hypoglycemia and islet-cell tumor. Adrenal or pituitary insufficiency and hepatic disease are relatively rare causes.

INSTRUCTOR: What tests would you advise?

STUDENT: Since the cause of the hypoglycemia is probably pancreatic, I would test the effect of ingested carbohydrate on the blood sugar level. The comparative levels of the fasting and the four-hour postprandial blood sugars are significant. In functional hypoglycemia, the fasting blood sugar is usually at a relatively normal level but the blood sugar two to four hours after meals is low, as the islet cells over-react to ingested carbohydrate. If there is an organic cause for the hyperinsulinism, the fasting blood sugar level is low and the blood sugar after meals is either elevated, normal or low, depending upon the time after eating.

The effect of a low carbohydrate diet is another differentiating test. If the hypoglycemia is functional, such a diet should restore the blood sugar level toward normal. If it is organic in origin—that is, if it is due to an islet-cell tumor or to hyperplasia—the reduced carbohydrate intake usually does not raise the blood sugar.

INSTRUCTOR: As you say, tumors are usually independent of physiologic regulation and the failure to respond to a low carbohydrate diet is the most important test for the identification of islet-cell tumors. The responses to glucose and insulin have been determined and are shown in Table 1. What features of these tests speak for or against an islet-cell tumor?

STUDENT: There are a number of very low fasting blood sugar values and they are suggestive of organic disease. Moreover, the failure of the blood sugar level (34, 35, 48 mg. per 100 ml.) to rise significantly after a low carbohydrate diet is strong evidence of a secreting tumor of the islets. Is it necessary to carry out any of the other tests?

INSTRUCTOR: For practical purposes the responses to glucose, insulin

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[1] In case it may be thought that this was *some* student, it is only fair to point out that his remarks were edited for the sake of conciseness.

TABLE 1 (Mrs. H.L.)

Date	Hour* ⟶	Blood glucose								
		0	1/4	1/2	1	2	3	4	5	6
		mg. per 100 ml.								
Sept. 1	Glucose tolerance	20		107	133	134	70	45	41	37
2	11:30 A.M.	60	30	15	17	Insulin tolerance				
	8:30 P.M.	71	74	74	75	Adrenalin tolerance				
4		26 162	Before glucose—EEG abnormal After glucose—EEG normal							
9 10 11		34 48 35	On these days patient was on a low carbohydrate diet (P-90, F-90, C-100)							
12 13 Sept. 14		16 26 25	Islet-cell tumor removed							

* Time 0 means the morning blood sugar concentration after an overnight fast, except when otherwise specified.

and adrenalin are of little or no use in distinguishing the type of hypoglycemia.[2]

[2] First, the insulin test was made by giving 0.1 unit per Kg. intravenously after the initial blood sample had been taken. Sensitivity to insulin often refers to different things. From an initial level of 60 mg., the blood sugar level fell to 15 mg. per 100 ml. This is an approximately normal sensitivity or response, although the interpretation of such a response at subnormal blood sugar levels is difficult or impossible. The words "sensitivity to insulin" are occasionally used to refer to the development of symptoms. This patient had no symptoms during this test, meaning that her nervous system was less sensitive to insulin (hypoglycemia) than usual. This ability to withstand low blood sugar levels is characteristic of many patients with islet-cell tumor; it is also seen in many diabetic patients who have taken large doses of insulin and in schizophrenic patients who require large doses of insulin for shock treatment. It is interesting to observe this response, but in my opinion the insulin test is not indicated as a regular procedure in these patients.

The response to adrenalin is a diagnostic aid in the hypoglycemia of glycogen storage disease (von Gierke), which occurs in children and is accompanied by an enlarged liver and acetonuria. In an adult without these findings, the failure of the blood sugar level to rise merely means that the liver glycogen was low at the moment of the test.

The glucose tolerance test may be of value in inducing hypoglycemia, especially at the fourth hour, in patients whose fasting blood sugar level is above 50 mg. per 100 ml., and who have not been observed in attacks.

As stated in Table 1, an islet-cell tumor was readily found and removed. Can you think of any precautions that the surgeon should take in exploring such a patient?

STUDENT: In 10 per cent of cases operated upon, more than one tumor is found; these may even occur in aberrant pancreatic tissue (2). Therefore, not only the entire pancreas, but also the duodenal wall, spleen and mesentery should be carefully searched for tumor.

INSTRUCTOR: Correct, and one might add that severe hypoglycemia should be avoided during anesthesia when the symptoms thereof are masked. This patient received intravenous glucose during the operation.

After operation all blood sugar levels were entirely normal and she made an uneventful recovery.

STUDENT: Was the electroencephalogram (EEG) obtained, in order to rule out some other cause of hypoglycemia?

INSTRUCTOR: No. This examination for the epileptic tendency is commonly of value in patients with neurologic symptoms. The extremely low blood sugar concentrations in this patient made the diagnosis apparent. It was, however, reassuring to know that when she had normal blood sugar levels, there were no stigmata of epilepsy. This was an indication that a good response ought to follow control of the hypoglycemia. The pattern of the EEG in hypoglycemia and in epilepsy is essentially the same. Patients with epilepsy are unduly sensitive to a lowering of the blood sugar level. Thus, diabetics with epilepsy may have insulin reactions at levels of 60 or 70 mg. per 100 ml. The same thing may be observed in instances of mild spontaneous hypoglycemia. An example of this occurred within the past year in this hospital.

Mrs. S., aged 30, was admitted in March 1953 to the Hospital of the University of Pennsylvania (Service of Dr. I. S. Ravdin). She had symptoms of gallbladder disease and roentgen examination showed calcification in the gallbladder. Preoperative studies revealed several slightly low values for blood sugar; *e.g.*, before lunch, 58 mg. and before breakfast, 64 and 70 mg. per 100 ml. For the previous four years she had had a history of occasional convulsions which were attributed to hypoglycemia. At operation cholelithiasis was found, the gallbladder was removed and, incidentally, the pancreas was explored but no tumors or other abnormalities were seen. Before leaving the hospital she had a "seizure," at which time the concentration of blood sugar was 102 mg. per 100 ml. Later, an EEG, when the blood sugar level was 99 mg. per 100 ml., showed a definite epileptiform alteration.

In such a situation the dietary regimen for functional hypoglycemia would be combined with suitable hydantoin therapy, in order to obtain the best results.

STUDENT: I have read that a low carbohydrate diet prevents or restores the low blood sugar in so-called functional hypoglycemia. How effective is it in such patients?

INSTRUCTOR: Only one who has seen the striking symptomatic improvement in these patients can appreciate the potency of the test by a low carbohydrate diet. Figure 1 illustrates this test and compares the response of normal persons (taken from Conn (3)), and of a severely neurasthenic

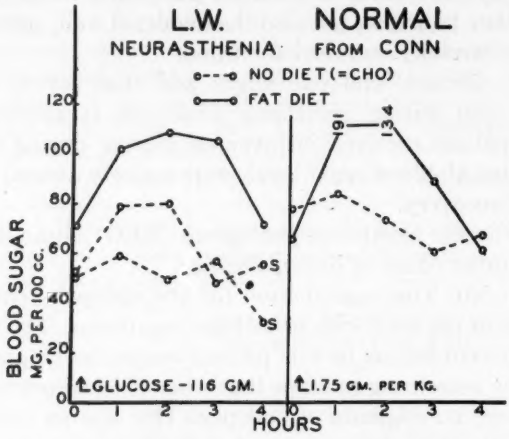

FIG. 1. Showing the response to glucose tolerance tests under various conditions. On the right are the mean values reported in Conn's (3) study of the effect of the antecedent ingestion of high and low carbohydrate diets on the sugar tolerance test. It is now well established that a low carbohydrate diet causes some elevation of the blood sugar levels in normal subjects. On the left are three curves on a patient, L.W., who had functional hypoglycemia. After an unrestricted diet which was high in carbohydrate, symptoms (S) of hypoglycemia developed at the fourth hour. On a fat diet (low carbohydrate) she was completely relieved of symptoms within forty-eight hours and had the elevated curve shown by the solid line.

patient (L.W.). Mrs. L.W. was referred because of extreme nervousness, manifested physically by uncontrolled choreiform movements and mentally by emotional instability and erratic behavior. Both physical and mental difficulties were completely relieved by the low carbohydrate regimen, which restored her blood sugar to normal levels.

Figure 2 shows the responses of the blood sugar in a patient (C.F.) with functional hypoglycemia, compared with those in another patient (J.R.) who had a proven islet-cell tumor. It should be noted that C.F. was treated almost twenty years ago, before the use of these tests was understood. Recalling these events is a forceful way of emphasizing the improvement in present-day diagnosis. Even this fails in some instances. In particular, there are cases of hypoglycemia in children which defy our best efforts at diagnosis and fail to yield to medical or surgical treatment (4).

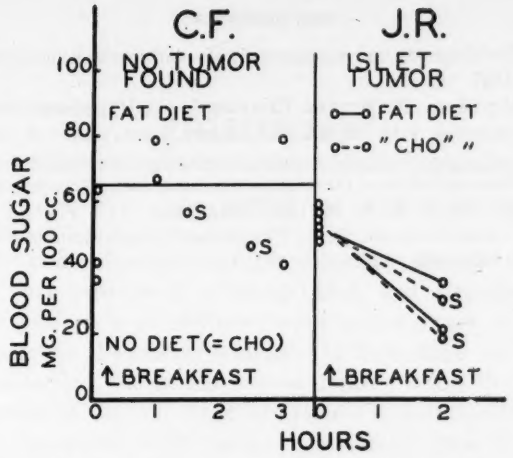

FIG. 2. Showing the blood sugar values, fasting and from one to three hours after breakfast in 2 patients with hypoglycemia. Results obtained when the patients took the unrestricted hospital diet (high carbohydrate) are indicated by the broken lines in the case of J.R. and by the values below the horizontal line at 63 mg. per 100 ml. in the case of C.F. In both, the "fat diet" was a low-carbohydrate diet (80 Gm. daily) which was high in fat, to meet caloric requirements.

In J.R., the change in diet led to no significant change in the behavior of the blood sugar. In view of this lack of response, islet-cell tumor was suspected and was found at operation, as previously reported (2).

In C.F., the low blood sugar levels after fasting overnight, and the fairly severe symptoms led to exploration of the pancreas, at which operation no tumor was found. During the period of postoperative reaction, the concentration of blood sugar was normal (as is the case, even in patients with islet-cell adenomas). After an uneventful convalescence, hypoglycemia reappeared and the patient was given the low carbohydrate diet (fat diet). Under this regimen his blood sugar values did not fall below 63 mg. per 100 ml. and his symptoms were entirely relieved.

In most adults, however, one can make a workable diagnosis between functional hypoglycemia and islet-cell adenoma. Under these circumstances treatment by diet or surgery, as indicated, is usually satisfactory.

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Editorial

STIMULATION OF GROWTH IN SHORT CHILDREN

THE clinical endocrinologist is often asked by parents or by patients, "Is there any treatment available that will stimulate growth in height?" If the patient is an obvious hypothyroid dwarf, with or without childhood myxedema, the answer is simple. In these cases the bone maturation is greatly retarded, the height is considerably below the normal range, and administration of thyroid substance results in a dramatically increased rate of growth. However, such cases are relatively rare. The more usual situation is that the patient comes from short parents who would like him to be taller than they, and thus possibly avoid some of their embarrassing experiences; or that the child is simply in the lower range of normal but decidedly below the average—being the shortest in the class at school, or shorter than a younger sibling, resulting in psychologic problems in the home.

There is some difference of opinion concerning what may be accomplished. In pituitary dwarfism, Shorr *et al.* (1) recently showed that large doses of growth hormone will stimulate growth; however, this material was not generally available, and frequent injections were required over a considerable period of time. In a similar case, Escamilla and Bennett (2) showed that there is an apparent synergistic effect when thyroid and methyltestosterone are given together, resulting in a stimulation of the rate of growth exceeding considerably that obtained with either substance alone, apparently without advancing the bone age. For the short child, many pediatricians give the conservative advice that no therapy is necessary. The patient and parents are simply "patted on the back" and told that more growth will occur later. However, if the physician also sees adults in practice, he is confronted with some frequency by short individuals who have reached the age of 21 cr 22 with epiphyses completely fused, who have been given such advice at an earlier age. These patients may be extremely desirous of achieving a greater height, but unfortunately they must be told that it is too late even to make an attempt.

It is the opinion of this observer that in some, although not in all short children, growth may be stimulated by the administration of thyroid and methyltestosterone to a height beyond that which would be reached without treatment. Therapy must be given while the epiphyses are still open,

and it has not caused an unusual rate of epiphyseal closure. Should the bone age be two or three years behind the chronologic age, it may be safe to observe the patient without treatment for six months to one year—particularly if the pubertal growth spurt has not yet occurred. If treatment is decided upon, it may be given as follows: in boys, methyltestosterone tablets (10 mg. once daily) with thyroid (30 mg. ($\frac{1}{2}$ grain) twice daily), the latter gradually being increased to tolerance. The same dose may be tried in girls but they must be observed carefully for signs of virilism, including increased hair growth on the upper lip, slight huskiness of the voice, or prominence of the clitoris. Should any of these signs appear, the dose of methyltestosterone should be decreased or omitted. If a greater effect is desired and the patient is old enough to cooperate, sublingual or buccal tablets of methyltestosterone may be used. Estrogens should not be given, even in the female patient, as they increase the rapidity of bone maturation and are not as effective in stimulating growth. Good results with methyltestosterone alone in boys have been reported by Lisser (3).

In growth problems generally, it is preferable not to depend entirely upon a wrist roentgenogram for estimation of the bone age. Examination also should be made of the joints most concerned with growth of the extremities; these include the elbow, hand, hip, knee, ankle, foot, and occasionally the shoulder. Comparison with Hodges' chart (4) for estimation of bone age has proved satisfactory in this regard.

Untoward effects from this therapy must be considered. They are apparently minimal from the administration of thyroid, if it is kept at a dose level within the patient's tolerance; once it is stopped a normal thyroid seems rapidly to regain its previous level of function. Methyltestosterone may cause virilism in girls, in which case the dose should be decreased or the medication discontinued. The degree of stimulation of sexual development in boys is usually not excessive, although it may occasionally be a reason for diminishing the daily dose. The rare instance of development of gynecomastia in boys may necessitate reduction of the dose or even discontinuance of testosterone therapy.

When under proper supervision, this method of therapy is relatively safe, and offers the possibility of help to short young patients. However, if it is delayed too long, the epiphyses will fuse and the time will have passed when any aid can be offered.

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Questions and Answers

(Questions submitted at the 1953 Postgraduate Assembly of the Endocrine Society and the University of Minnesota)

Answers by Robert L. Noble, Univ. of Western Ontario, London, Ontario, Canada

QUESTION: Should asymptomatic hypercholesterolemia be treated?

ANSWER: This question is very debatable, since the role of hypercholesterolemia in the etiology of disease is still unsettled. It is essential first to rule out any of the known conditions affecting the blood cholesterol level.

QUESTION: Why does water retention occur with hypoadrenalism; instead, one would expect it to occur in hyperadrenalism, because of increased sodium retention stimulating the posterior lobe of the pituitary to secrete antidiuretic hormone?

ANSWER: Water retention should not be confused with the inability to excrete a water load; retention may be related to excess treatment by adrenal steroids, but it does not occur in adrenal insufficiency. Following adrenalectomy there may be an immediate diuresis related to sodium loss, but there rapidly develops an inability to excrete ingested water at the normal rate. As a result, practically no diuresis occurs within the first three hours. However the water is eventually lost, so that retention does not occur. Although the posterior lobe of the pituitary responds to changes in the osmotic pressure of the blood, its interrelationship with adrenal steroids is still not clearly defined.

QUESTION: Would you stimulate linear growth (height) in adolescent males by testosterone?

ANSWER: This hormone (particularly in the form of the slowly absorbed long-acting derivatives) has been used to stimulate growth and to cause nitrogen retention. Before such therapy is instituted a careful consideration of the etiology of the condition being treated and full appreciation of all actions of the hormone must be considered.

QUESTION: Do androgens stimulate growth? The eunuch is taller than normal.

ANSWER: Androgens have been used to stimulate growth under certain conditions. Secondary hormonal changes such as those caused by increased pituitary growth hormone may play a role in the case of the eunuch.

Answers by George W. Thorn, Peter Bent Brigham Hospital, Boston, Mass.

QUESTION: Is hydrocortisone available commercially?

ANSWER: Hydrocortisone is available commercially in both oral and saline suspension forms. It is made by Merck and Company under the name of Hydrocortone, and the tablets each contain 20 milligrams. In the suspension form (5-cc. vials) each cubic centimeter contains 25 milligrams. The Upjohn Company produces hydrocortisone under the name of Cortef in 10 and 20 milligram tablets and in 1 per cent and 2½ per cent topical ointment. Pfizer Company has made available both oral and ointment forms of hydrocortisone with the trade-name Cotril.

QUESTION: Does desoxycorticosterone have any place in the treatment of collagen disease?

ANSWER: In my opinion desoxycorticosterone has no place in the treatment of collagen disease.

Answer by Edmund B. Flink, Univ. of Minnesota Medical School, Minneapolis, Minn.

QUESTION: Has the Sulkowitch test been modified for quantitative estimation of calcium?

ANSWER: If the amount of calcium excreted in the urine is going to be determined, an accurate method is needed (the method published by A. C. Sobel and B. A. Sobel, Jr., *J. Lab. & Clin. Med.* **26**: 585, 1940, is a good one). The difference between normal and abnormal calcium excretion may be small, so only very accurate methods are adequate.

The Sulkowitch test is excellent as a qualitative test for the presence of excess calcium or the absence of calcium. It has been modified for semiquantitative determinations by means of turbidity measurement with a photoelectric colorimeter. However, the modification is subject to considerable error and it is judged to be unsatisfactory.

Answer by Robert H. Williams, Univ. of Washington School of Medicine, Seattle, Wash.

QUESTION: Can oral thyroid in a dose of 1 grain three times a day, and Dexedrine, 10.0 mg. twice a day, produce exophthalmos? (I saw a patient who had been receiving this medication for two years, in whom exophthalmos (2+) had developed near the end of this course of therapy. She improved to some extent when I stopped treatment.)

ANSWER: Because of the enormous number of times that this amount of thyroid and Dexedrine have been given without producing any changes in the eyes, the development of exophthalmos in this instance I regard as a coincidence. Many instances are known in which thyrotoxicosis, with or without significant eye changes, has developed while patients have been taking thyroid and has persisted for months or years following complete cessation of therapy. It has been conjectured that such therapy has upset the pituitary-thyroid axis. Thyrotoxicosis has occurred following cessation of thyroid therapy in other instances. I do not believe the Dexedrine played any role, except perhaps insofar as the extra excitement associated with its administration may have served as a trigger mechanism for setting off Graves' disease in a person who was already strongly predisposed.

Answer by Samuel F. Haines, Mayo Clinic, Rochester, Minn.

QUESTION: In order to determine the effectiveness of desiccated thyroid in the management of dry scaly skin or brittle nails, what dose should be given and for how long?

ANSWER: Ordinarily, in patients with myxedema, the effect of desiccated thyroid on the skin is noticeable within a few weeks after the institution of treatment. I do not believe that desiccated thyroid should be used because of dry scaly skin, or for brittle nails, unless there are evidences of myxedema, or unless the basal metabolic rate is significantly below the average normal level. Even in the latter case, if myxedema is not present, the effect of desiccated thyroid on the skin would be very questionable. I know of no good

evidence that thyroid function is in itself related to brittle nails. The dose of desiccated thyroid, when administered, should be worked out for each individual, an attempt being made to hold the basal metabolic rate somewhere near, but not above, the average normal level, with due consideration of the clinical status of the patient receiving it.

Answer by Gerald T. Evans, Univ. of Minnesota Medical School, Minneapolis, Minn.

QUESTION: Please discuss the problem of failure to gain weight in persons who eat well, have no infection, no signs of sprue or liver disease and no evidence of hypermetabolism.

ANSWER: To the best of my knowledge the thin overeater, who in essence is described in the question, has never been thoroughly investigated. This would necessitate the hospitalization and elaborate examination of well individuals for long periods, which probably explains why it has not been done. Most clinical students of nutrition believe the thin overeater really exists.

I have practically no experience with cases like those mentioned. Confronted with such a patient, who had real need to gain weight, I should like to 1) check the intake (hearsay is not good enough), 2) determine the dry weight of feces (under 50 Gm./day), and 3) secure evidence of a normal amount of activity, both asleep and awake. Cleared on all these points, I believe that most nutritionists would then, with a suitable diet, use small doses of insulin. Insulin, theoretically, promotes the conversion of carbohydrate to fat, which is the missing item in the make-up of the described individual. There have been reports of its successful use in such a way.

The Endocrine Society

THE 1954 ANNUAL MEETING

The Thirty-sixth Annual Meeting of The Endocrine Society will be held in the Sir Francis Drake Hotel, San Francisco, California, Thursday, Friday and Saturday, June 17–18–19, 1954.

Dr. Roberto F. Escamilla, 655 Sutter Street, San Francisco, California, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Sir Francis Drake Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 18, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

Hotel reservations may be made through the San Francisco Convention and Visitors' Bureau, Room 300, Civic Auditorium, San Francisco, California. They should be accompanied by the official AMA application form which appears during December of 1953 in JAMA.

Arrangements have been made for members of this society who do not belong to the AMA to be assigned rooms during the days of the meeting of the Endocrine Society. Applications from nonmembers of the AMA should specify dates of arrival and departure and be accompanied by a deposit of $10.00 per room.

Confirmations will not be sent until after February 1, 1954, but *applications should be made promptly* in order to insure the reservation.

1954 AWARD AND FELLOWSHIPS

Awards and Fellowships presented by The Endocrine Society carry no obligation by the recipient to the Society or to the donors.

Fellowships

Society Fellowships are designed to assist men or women of exceptional promise in furthering their educational training and advancement toward a scientific career in endocrinology. Fellowships may be awarded to an individual who possesses the Ph.D. or M.D. degree or to a candidate for either of these degrees. The stipend, which will not exceed $5,000 may be divided into two Fellowships in varying amounts, in accordance with the qualifications of the appointee. The Committee will, in reviewing the proposed program of study, consider the amount of time which the Fellow intends to

spend in course work and/or teaching. He must present evidence of scientific ability as attested by studies completed or in progress and/or the recommendation of responsible individuals. He must submit a program of proposed study, indicate one or more institutions where the proposed program will be followed, and submit statements of approval from the investigators with whom he proposes to conduct his research. He must serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be allotted for course work or for participation in teaching, the latter purely on a voluntary basis.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

The Ayerst, McKenna and Harrison Fellowship was first awarded in 1947 to Dr. Samuel Dvoskin and in 1948 to Dr. Ernest M. Brown, Jr. Dr. Brown was re-elected in 1949. The Fellowship in 1950 was awarded to Dr. Lawrence E. Shulman and in 1952 to Dr. John C. Laidlaw. In 1954 it will be in the amount of $5,000.

THE SCHERING FELLOWSHIP IN ENDOCRINOLOGY

This Fellowship will not be awarded in 1954 in order that it may accumulate toward a $5,000 Fellowship to be awarded in 1955.

Award

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishment of an investigator, not more than thirty-five years of age, in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; in 1945 to Dr. Jane A. Russell; in 1946 to Dr. Martin M. Hoffman; in 1947 to Dr. Choh Hao Li; in 1948 to Dr. Carl Heller; in 1949 to Dr. George Sayers; in 1950 to Dr. Oscar M. Hechter; in 1951 to Dr. Albert Segaloff. Effective in 1952, the Award was increased from $1,200 to $1,800 and the recipient was Dr. Seymour Lieberman; in 1953 the Award was presented to Drs. Sidney Roberts and Clara Szego. If within twenty-four months of the date of the Award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

Nominations

Each member has the privilege of making one nomination for each Fellowship or Award. A nomination should be accompanied by a statement of the importance of the nominee's contributions to, or interest in, endocrinology and by a bibliography of the nominee's most important publications, with reprints if possible. The nominations should be made on special application forms which may be obtained from the Secretary, Dr. Henry H. Turner, 1200 North Walker Street, Oklahoma City, Oklahoma, and returned to him not later than *March 1, 1954.*

The American Goiter Association

THE 1954 ANNUAL MEETING

The annual meeting will be held at the Somerset Hotel, Boston, Massachusetts, April 29, 30 and May 1, 1954. It is suggested that you make your hotel reservations by writing Mr. Harry Hoghn, Executive Assistant Manager, The Somerset, 400 Commonwealth Avenue, Boston 15, Massachusetts.

JOHN C. MCCLINTOCK, M.D.,
Corresponding and Recording Secretary